

UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
10441Z

Total Pages in this Submission
3

TO THE ASSISTANT COMMISSIONER FOR PATENTS

Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME

and invented by:

Nicholas Kim Hayward
Gunther Weber
Sean Grimmond

If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: 08/765,588

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: _____

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: _____

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 58 pages and including the following:
 - a. ☒ Descriptive Title of the Invention
 - b. ☐ Cross References to Related Applications (if applicable)
 - c. ☐ Statement Regarding Federally-sponsored Research/Development (if applicable)
 - d. ☐ Reference to Microfiche Appendix (if applicable)
 - e. ☒ Background of the Invention
 - f. ☒ Brief Summary of the Invention
 - g. ☒ Brief Description of the Drawings (if drawings filed)
 - h. ☒ Detailed Description
 - i. ☒ Claim(s) as Classified Below
 - j. ☒ Abstract of the Disclosure

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Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

- a. ☐ Formal Number of Sheets _____
- b. ☒ Informal Number of Sheets 52

4. ☒ Oath or Declaration

- a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
- b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
- c. ☒ With Power of Attorney ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*

The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche *(Appendix)*

7. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*

- a. ☐ Paper Copy
- b. ☐ Computer Readable Copy *(identical to computer copy)*
- c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & document(s))*

9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☒ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

☐ First Class ☒ Express Mail *(Specify Label No.):* EL087018078US

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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)

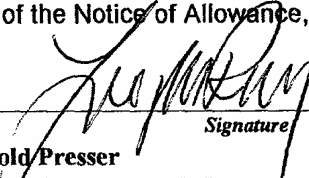
16. ☐ Additional Enclosures (please identify below):

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	5 *	- 20 =	0	x \$18.00	\$0.00
Indep. Claims	2 *	- 3 =	0	x \$78.00	\$0.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
* Claims calculated based on Preliminary Amendment filed herewith BASIC FEE					\$760.00
OTHER FEE (specify purpose)					\$0.00
TOTAL FILING FEE					\$760.00

- ☒ A check in the amount of \$760.00 to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 19-1013/SSMP as described below. A duplicate copy of this sheet is enclosed.
- ☐ Charge the amount of _____ as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).


Signature

Leopold Presser
Registration No.: 19,827

Dated: July 8, 1999

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cc:

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Nicholas K. Hayward, et al. **Examiner:**
Serial No.: Unassigned **Art Unit:**
Filing Date: Herewith **Docket:** 10441Z
For: A NOVEL GROWTH FACTOR AND A **Date:** July 8, 1999
 GENETIC SEQUENCE ENCODING SAME

Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination, please amend the above-identified patent application as follows:

IN THE SPECIFICATION:

Page 1, before line 5, please insert the following:

--CROSS REFERENCE TO RELATED APPLICATION

The present application is a continuation of application Serial Number 08/765,588, filed April 25, 1997.--

IN THE CLAIMS:

Please cancel Claims 1-25, 29, 31-42 without prejudice.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

"Express Mail" mailing label number: EL087018078US
Date of Deposit: July 8, 1999

I hereby certify that this New Patent Application and Fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, DC 20231.

Dated: July 8, 1999


Michelle Spina

Please amend the claims as follows:

26. (Amended) An isolated nucleic acid [molecule comprising a sequence of nucleotides or complementary to a sequence encoding a proteinaceous molecule having the following characteristics:

(i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2; (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF)] encoding or complementary to a sequence encoding a polypeptide comprising an amino acid sequence having at least about 70% similarity to SEQ ID NO:6.

Claim 27, lines 1 and 2, delete "the proteinaceous molecule" and insert therefor --said polypeptide--.

Claim 28, lines 1 and 2, delete "the proteinaceous molecule" and insert therefor --said polypeptide--.

Claim 30, line 1, delete "1" and insert therefor --26--.

Claim 30, line 1, delete "molecule" both occurrences and insert --nucleic acid-- after "said".

Please add the following new claims:

--43. A process for the production of a biologically active VEGF-like protein comprising expressing a nucleic acid molecule encoding a polypeptide having at least about 70% similarity to SEQ ID NO:6 in a host and isolating said VEGF-like protein.

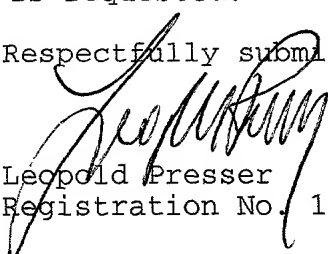
44. The method according to Claim 43 wherein said nucleic acid comprises SEQ ID NO:5 or has at least about 70% similarity to SEQ ID NO:5 or hybridizes under low stringency conditions to a reverse complement of the nucleotide sequence of SEQ ID NO:5.--

REMARKS

Applicants have amended the specification to insert cross-reference to a related application Serial Number 08/765,588, filed April 25, 1997. Claims 26-28 and 30 have been amended. Support for the amendment to Claim 26 may be found throughout the specification and particularly at Page 3, lines 4-10, for example. Claims 27, 28 and 30 have been amended to reflect proper dependency. Claims 43-44 have been added to further define the subject matter to which applicants are entitled. Support for Claims 43-44 may be found throughout the specification and particularly at originally filed Claims 35, 36 and 39 and Page 14, Table 1 of the specification. No new matter has been added.

It is respectfully requested that this Preliminary Amendment be entered in this application prior to examination. Early and favorable consideration is requested.

Respectfully submitted,


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A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE
ENCODING SAME

5 The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present
10 invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for
15 the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to
20 imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Vascular endothelial growth factor (hereinafter referred to as "VEGF"), also known as vasoactive permeability factor, is a secreted, covalently linked homodimeric glycoprotein
25 that specifically activates endothelial tissues (Senger *et al.*, 1993). A range of functions have been attributed to VEGF such as its involvement in normal angiogenesis including formation of the corpus luteum (Yan *et al.*, 1993) and placental development (Sharkey *et al.*, 1993), regulation of vascular permeability (Senger *et al.*, 1993), inflammatory angiogenesis (Sunderkotter *et al.*, 1994) and autotransplantation (Dissen *et al.*, 1994) and
30 human diseases such as tumour promoting angiogenesis (Folkman & Shing, 1992), rheumatoid arthritis (Koch *et al.*, 1994) and diabetes related retinopathy (Folkman & Shing, 1992).

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VEGF is, therefore, an important molecule making it a potentially valuable target for research into therapeutics, prophylactics and diagnostic agents based on VEGF or its activities. There is also a need to identify homologues or otherwise related molecules for use as an alternative to VEGF or in conjunction with VEGF.

5

In work leading up to the present invention, the inventors sought the multiple endocrine neoplasia type I susceptibility gene (MEN1). Surprisingly, the inventors discovered that a genetic sequence excluded as a candidate for the MEN1 gene was nevertheless a new growth factor having some similarity to VEGF. Furthermore, the growth factor of the present invention is an effector molecule for primary and central neurons.

10

Accordingly, one aspect of the present invention comprises a biologically isolated proteinaceous molecule comprising a sequence of amino acids which:

- (i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and
- (ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

15

Another aspect of the present invention provides a biologically isolated proteinaceous molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with VEGF.

20

A related aspect of the present invention contemplates a biologically isolated proteinaceous molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one of the following properties:
 - (a) ability to induce proliferation of vascular endothelial cells;
 - (b) ability to interact with *flt-1/flk-1* family of receptors;

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- (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

By "biologically isolated" is meant that the molecule has undergone at least one step of purification from a biological source. Preferably, the molecule is also biologically pure meaning that a composition comprises at least about 20%, more preferably at least about 40%, still more preferably at least about 65%, even still more preferably at least about 80-90% or greater of the molecule as determined by weight, activity or other convenient means, relative to other compounds in the composition. Most preferably, the molecule is sequencably pure.

Another preferred aspect of the present invention provides the molecule in recombinant form.

According to this aspect of the present invention, there is provided a recombinant molecule comprising a sequence of amino acids which:

- (i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and
- (ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

20

A related aspect of the present invention is directed to a recombinant molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with VEGF.

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A further related aspect of the present invention contemplates a recombinant molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;

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(ii) exhibits at least one of the following properties:

- (a) ability to induce proliferation of vascular endothelial cells;
- (b) ability to interact with *flt-1/flk-1* family of receptors;
- (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

The present invention also contemplates genomic or partial genome clones encoding a proteinaceous molecule having at least about 15% amino acid similarity but at least about 5% dissimilarity to SEQ ID NO:1.

The amino acid sequence set forth in SEQ ID NO:2 corresponds to human VEGF (referred to herein as "VEGF₁₆₅"). Accordingly, the molecule of the present invention is VEGF-like or is a homologue of VEGF but comprises an amino acid sequence which is similar but non-identical to the amino sequence of VEGF. Although the present invention is exemplified using a human VEGF-like molecule, this is done with the understanding that the instant invention contemplates the homologous molecule and encoding sequence from other mammals such as livestock animals (e.g. sheep, pigs, horses and cows), companion animals (e.g. dogs and cats) and laboratory test animals (e.g. mice, rats, rabbits and guinea pigs) as well as non-mammals such as birds (e.g. poultry birds), fish and reptiles. In a most preferred embodiment, the VEGF-like molecule is of human origin and encoded by a gene located at chromosome 11q13. The present invention extends, therefore, to the genomic sequence or part thereof encoding the subject VEGF-like molecule.

Preferably, the percentage similarity is at least about 30%, more preferably at least about 40%, still more preferably at least about 50%, still even more preferably at least about 60-70%, yet even more preferably at least about 80-95% to all or part of the amino acid sequence set forth in SEQ ID NO:2.

In a particularly preferred embodiment, the VEGF-like molecule of the present invention comprises a sequence of amino acids as set forth in SEQ ID NO:4 or is a part, fragment, derivative or analogue thereof. Particularly preferred similarities include about 19-20%,

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and 29-30%. Reference herein to derivatives also includes splice variants. Accordingly, the present invention extends to splice variants of SOM175. Examples of splice variants contemplated by the present invention include but are not limited to variants with an amino acid sequence substantially as set forth in at least one of SEQ ID NO:6, SEQ ID
5 NO:8 and/or SEQ ID NO:10 or mutants or derivatives or further splice variants thereof.

Another embodiment provides a recombinant molecule having the following characteristics:

- 10 (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

15 Another embodiment provides a recombinant molecule having the following characteristics:

- 20 (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

Another embodiment provides a recombinant molecule having the following characteristics:

- 25 (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

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Another embodiment provides a recombinant molecule having the following characteristics:

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- (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- 5 (ii) exhibits at least one biological property in common with VEGF.

Such properties of VEGF include at least one of:

- (a) ability to induce proliferation of vascular endothelial cells;
- (b) an ability to interact with *flt-1/flk-1* family of receptors;
- 10 (c) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

In accordance with the present invention, a preferred similarity is at least about 40%, more preferably at least about 50% and even more preferably at least about 65% similarity.

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Still a further aspect of the present invention contemplates a peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a splice variant thereof such as set forth in SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 or a chemical equivalent thereof. The biologically isolated or recombinant molecule of the present invention may be naturally glycosylated or may comprise an altered glycosylation pattern depending on the cells from which it is isolated or synthesised. For example, if produced by recombinant means in prokaryotic organisms, the molecule would be non-glycosylated. The molecule may be a full length, naturally occurring form or may be a truncated or otherwise derivatised form.

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Yet another aspect of the present invention is directed to a nucleic acid molecule encoding the VEGF-like molecule herein described. More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or having at least 15% similarity to all or part thereof or being capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the

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nucleic acid sequence having at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence as set forth in SEQ ID NO:3. The nucleotide sequence set forth in SEQ ID NO:3 is also referred to herein as "SOM175". Preferably, the percentage dissimilarity is about 35%, more preferably about 39% and even more preferably about 40-50% or greater.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al* (1989) at pages 9.47-9.51 which is herein incorporated by reference where the washing steps disclosed are considered high stringency. A low stringency is defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at $\geq 45^{\circ}\text{C}$ for 2-3 hours or high stringent conditions considered herein to be 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

The present invention further contemplates a nucleic acid molecule which encodes a VEGF-like molecule as hereinbefore described having at least 15% nucleotide sequence homology to SEQ ID NO:3. Preferred levels of homology include at least about 40%, more preferably around 60-70%.

The present invention is further directed to the murine homologue of human VEGF (referred to herein as "mVRF"). The mVRF has approximately 85% identity and 92% conservation of amino acid residues over the entire coding region compared to human VEGF. The mVRF is encoded by a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in Figure 9.

The VEGF-like molecule of the present invention will be useful in the development of a range of therapeutic and/or diagnostic applications alone or in combination with other molecules such as VEGF. The present invention extends, therefore, to pharmaceutical compositions comprising the VEGF-like molecule or parts, fragments, derivatives, homologues or analogues thereof together with one or more pharmaceutically acceptable

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carriers and/or diluents. Furthermore, the present invention extends to vectors comprising the nucleic acid sequence set forth in SEQ ID NO:3 or having at least about 15%, more preferably about 40% and even more preferably around 60-70% similarity thereto but at least 30% and more preferably around 39% dissimilarity thereto and host cells comprising same. In addition, the present invention extends to ribozymes and antisense molecules based on SEQ ID NO:3 as well as neutralizing antibodies to the VEGF-like molecule. Such molecules may be useful in ameliorating the effects of, for example, over expression of VEGF-like genes leading to angiogenesis or vascularization of tumours.

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Another aspect of the present invention contemplates a method of inducing astroglial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

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Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:6.

25 A further aspect of the present invention provides a method of promoting neural survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

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said administration being for a time and under conditions sufficient to induce astroglial proliferation.

Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence
5 set forth in SEQ ID NO:3 or SEQ ID NO:6.

The present invention also contemplates antibodies to the VEGF-like molecule or nucleic acid probes to a gene encoding the VEGF-like molecule which are useful as diagnostic agents.

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The present invention is further described by reference to the following non-limiting Figures and/or Examples.

In the Figures:

15

Figure 1 Nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence [SEQ ID NO:2] of VEGF₁₆₅.

Figure 2 Nucleotide sequence [SEQ ID NO:3] and corresponding amino acid
20 sequence [SEQ ID NO:4] of SOM175.

Figure 3 Results of BLAST search with SOM175 protein sequence.

Figure 4 BESTFIT alignment of VEGF cDNA and SOM175 cDNA.

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Figure 5 Multiple alignment of VEGF₁₆₅ with SOM175 and its splice variants at the nucleotide level.

Figure 6 Multiple alignment of VEGF₁₆₅ with SOM175 and its splice variants at
30 the amino acid level.

Figure 7 Diagrammatic representation of SOM175 and its splice variants.

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Figure 8(a) Diagrammatic representation of genomic structure of human SOM175 genomic showing exon/intron map.

Figure 8(b) Diagrammatic representation of genomic structure of human SOM175 showing exon/intron boundaries.

Figure 9 Nucleotide and predicted peptide sequences derived from mVRF cDNA clones. Numbering of nucleotides are given on the left, starting from the A of the initiation codon. Amino acids are numbered on the right, starting from the first residue of the predicted mature protein after the putative signal peptide has been removed. The alternately spliced region is double underlined and the resulting peptide sequence from each mRNA is included. A potential polyadenylation signal is indicated in boldface. Start and stop codons of mVRF₁₆₇ and mVRF₁₈₆ are underlined and a polymorphic AC repeat in the 3' UTR is indicated by a stippled box. The positions of intron/exons boundaries are indicated by arrowheads.

Figure 10 BESTFIT alignments of human and murine VRF protein isoforms. A: mVRF₁₆₇ and hVRF₁₆₇. B: mVRF₁₈₆ and hVRF₁₈₆ from the point where the sequences diverge from the respective 167 amino acid isoforms. Amino acid identities are marked with vertical bars and conserved amino acids with colons. An arrow marks the predicted signal peptide cleavage site of human and mouse VRF.

Figure 11 BESTFIT alignment of mVRF₁₆₇ and mVEGF₁₈₈ (Breier *et al*, 1992) peptide sequences. An arrow marks the signal peptide cleavage site of mVEGF. Identical amino acids are indicated by vertical bars and conservative substitutions by colons. Numbering of amino acids is as described in the legend to Figure 9.

Figure 12 Comparison of gene structure between VRF (a generic VRF gene is shown since the intron/exon organisation of the mouse and human homologues is almost identical) and other members of the human VEGF/PIGF/PDGF gene family. Exons are represented by boxes. Protein coding regions and untranslated regions are shown by filled and open sections respectively. The hatched region in VRF indicates the

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additional 3' UTR sequence formed by alternate splicing of the VRF₁₈₆ isoform. Potential alternate splice products of each gene are shown.

Figure 13 Autoradiogram of a Northern blot of total RNA from various adult mouse tissues (as indicated) hybridised with an mVRF cDNA clone. A major transcript of 1.3 kb was detected in all samples.

Figure 14 Film autoradiographs (A-C) and dark-field micrographs (D-E) illustrating the expression pattern of mVRF and mRNA in the mouse. In the E14 mouse embryo (A) positive signals are present over the developing heart (Ha) and cerebral cortex (Cx). A low background signal is also present over other tissues in the section. In the E17 embryo (B) and the heart (Ha) is clearly visible due to a strong hybridisation signal. An equally strong signal is present over brown adipose tissue (Fa) in the back and around the thoracic cage. A moderate hybridisation signal is present over the spinal cord (SC) and the tongue (T). The background signal is reduced compared with the E14 embryo. In the young adult mouse (C-D), positive signals are present over the heart (Ha) and adipose tissue (Fa) around the thoracic cage, while, for example, the lungs (Lu) are unlabeled). The hybridisation signal over the heart is evenly distributed over the entire left ventricle, including papillary muscles (D). In the E17 heart hybridised with an excess of cold probe, no positive signal is present (E). Scale bars = 0.5 mm (A), 1.2 mm (B), 1 mm (C), 0.3 mm (D), 0.1 mm (E).

Figure 15 Dark - (A and C) and bright-field (B and D) micrographs showing mVRF mRNA expression in mouse adipose tissue (A-B) and spinal cord (C-D). A strong hybridisation signal is present over fat (A), as shown by the strong labeling in Sudan black stained sections (B). A weak signal is present also in skeletal muscle (M in A-B). In the adult spinal cord (C) the mVRF probes gave a neuronal staining pattern over the gray matter. Toluidine counterstaining showing that motoneurons in the ventral horn (D), interneurons in the deep part of the dorsal horn and around the central canal (not shown) where largely positive for mVRF mRNA. Scale bars = 0.1 mm (A), 0.1 mm (B), 0.25 mm (C), 0.015 mm (D).

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Figure 16 Effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival, % neurite outgrowth and average neurite length (μm).

Figure 17 Effects of VEGF and SOM175 on chick glia. Tested were CNS glial,
5 peripheral glia and CNS oligodendrocytes.

Figure 18 Effect of various SOM175 proteins on mouse astroglial cells. ■ ^3H (cpm)

- | | | |
|----|-----|--|
| | 1. | FGF-2 (10 ng/ml) positive control |
| | 2. | SOM Δ X6* 1 ng/ml |
| 10 | 3. | SOM Δ X6 10 ng/ml |
| | 4. | SOM Δ X6 100 ng/ml |
| | 5. | SOM Δ X6 1000 ng/ml |
| | 6. | SOM Δ X6 1000 ng/ml, no heparin |
| | 7. | SOMX6** 1 ng/ml |
| 15 | 8. | SOMX6 10 ng/ml |
| | 9. | SOMX6 100 ng/ml |
| | 10. | SOMX6 1000 ng/ml |
| | 11. | SOMX6 1000 ng/ml, no heparin |

* This refers to SOM175 absent exon 6;

20 ** This refers to SOM175.

Figure 19 Effect of various SOM175 proteins on mouse oligodendroglial cells. ■ ^3H (cpm)

- | | | |
|----|----|--|
| | 1. | FGF-2 (10 ng/ml) positive control |
| 25 | 2. | SOM Δ X6* 1 ng/ml |
| | 3. | SOM Δ X6 10 ng/ml |
| | 4. | SOM Δ X6 100 ng/ml |
| | 5. | SOM Δ X6 1000 ng/ml |
| | 6. | SOM Δ X6 1000 ng/ml, no heparin |
| 30 | 7. | SOMX6** 1 ng/ml |
| | 8. | SOMX6 10 ng/ml |
| | 9. | SOMX6 100 ng/ml |

- 13 -

10. SOMX6 1000 ng/ml
11. SOMX6 1000 ng/ml, no heparin

* This refers to SOM175 absent exon 6;

** This refers to SOM175.

5

Figure 20 Effect of various SOM175 proteins on mouse forebrain neurons. ■ % survival

- | | | |
|----|-----|--|
| | 1. | FGF-2 (10 ng/ml) positive control |
| | 2. | SOM Δ X6* 1 ng/ml |
| 10 | 3. | SOM Δ X6 10 ng/ml |
| | 4. | SOM Δ X6 100 ng/ml |
| | 5. | SOM Δ X6 1000 ng/ml |
| | 6. | SOM Δ X6 1000 ng/ml, no heparin |
| | 7. | SOMX6** 1 ng/ml |
| 15 | 8. | SOMX6 10 ng/ml |
| | 9. | SOMX6 100 ng/ml |
| | 10. | SOMX6 1000 ng/ml |
| | 11. | SOMX6 1000 ng/ml, no heparin |

* This refers to SOM175 absent exon 6;

20 ** This refers to SOM175.

553020-1466460

TABLE 1
SUMMARY OF SEQUENCE IDENTITY NUMBERS

5	SEQ ID NO:1	Nucleotide sequence of VEGF ₁₆₅
	SEQ ID NO:2	Amino acid sequence of VEGF ₁₆₅
	SEQ ID NO:3	Nucleotide sequence of SOM175 (VEGF-like molecules)
	SEQ ID NO:4	Amino acid sequence of SOM175
10	SEQ ID NO:5	Nucleotide sequence of SOM175 absent exon 6
	SEQ ID NO:6	Amino acid sequence of SOM175 absent exon 6
	SEQ ID NO:7	Nucleotide sequence of SOM175 absent exon 6 and exon 7
	SEQ ID NO:8	Amino acid sequence of SOM175 absent exon 6 and exon 7
	SEQ ID NO:9	Nucleotide sequence of SOM175 absent exon 4
15	SEQ ID NO:10	Amino acid sequence of SOM175 absent exon 4
	SEQ ID NO:11	Oligonucleotide
	SEQ ID NO:12	Oligonucleotide
	SEQ ID NO:13	Oligonucleotide
	SEQ ID NO:14	Oligonucleotide

EXAMPLE 1

Human cDNA clones

The original SOM175 cDNA was isolated by screening a human foetal brain library
 25 (λzapII, Stratagene) with the cosmid D11S750 (Larsson *et al*, 1992). The plasmid was
 excised "*in vivo*" and a single 1.1kb cDNA was obtained. Three independent SOM175
 cDNAs clones were also isolated from a human foetal spleen library (Stratagene, Uni-
 zap) using the above-mentioned SOM175 insert as a probe. Three clones were obtained:
 SOM175-4A, -5A and -6A. SOM175-5A is an alternately spliced clone with exon 4
 30 being absent (SOM175-e4). These library screens were performed using hybridisation
 conditions recommended by the manufacturer of the library (Stratagene) and random
 primed insert of SOM175.

- 15 -

Two partial human SOM175 cDNAs have also isolated from a λ GT11 human melanoma cell line A2058 library (Clontech) cDNA library screens were performed using hybridisation conditions described by Church and Gilbert, 1984). In each case, the probe was generated by random priming of a PCR product derived from SOM175 (18f-
5 700r).

Mouse cDNA Clones

Human SOM175 was also used to screen a mouse neonatal whole brain cDNA library (Unizap, Stratagene). Four non-chimeric clones were isolated: M175-A, B, C, D. All
10 clones were partial cDNAs and M175-C contained several introns. Three of these cDNAs lacked the exon 6.

Another clone referred to as M1 was completely sequenced and was found to contain the full open reading frame plus part of the 5'utr and total 3'utr.
15

EXAMPLE 2

DNA SEQUENCE ANALYSIS

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in Figure 2 with its corresponding amino acid sequence. This sequence was screened for
20 open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed (see Figure 2). There appears to be little 5' untranslated sequences (2bp). The 3' untranslated region appears to be complete as it includes a poly-adenylation signal and poly-A tail.

25 Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see Figure 3). The amount of homology between SOM175 and human VEGF₁₆₅ was determined using the BESTFIT program (GCG, University of Wisconsin; see Figures 4 and 5). Nucleotide homology was estimated at 69.7% and protein homology was
30 estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams *et al.*, 1993).

- 16 -

These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47, 5 Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in Figure 6. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has 10 unique properties of its own. The nucleotide sequence and corresponding amino acid sequence of VEGF₁₆₅ is shown in Figure 1.

EXAMPLE 3

The percentage similarity and divergence between VEGF₁₆₅ family and SOM175 family 15 (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 2.1 and 2.2. The alternatively spliced forms of SOM175 are abbreviated to SOM715-e6 where all of exon 6 is deleted; SOM715-e6 and 7 where all of exons 6 and 7 are deleted; and SOM175-e4 where all of exon 4 is deleted. The spliced form of SOM175 are shown in Figure 7. Genomic 20 maps of SOM175 showing intron/exon boundaries are shown in Figure 8a and 8b.

- 17 -

Table 2.1

A Percent nucleotide similarity between splice variants of SOM175 and
 5 human VEGF₁₆₅

	VEGF ₁₆₅	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
10 VEGF ₁₆₅	***	34.9	39.7	41.4	37.0
SOM175		***	98.9	95.1	99.2
SOM175-e6			***	98.8	84.0
SOM175-e6&7				***	80.3
SOM175-e4					***

15

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- 18 -

B Percent nucleotide divergence between splice variants of SOM175 and human VEGF₁₆₅

5		VEGF ₁₆₅	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
	VEGF ₁₆₅	***	41.7	41.6	41.7	41.8
	SOM175		***	0.2	0.2	0.0
	SOM175-e6			***	0.0	0.2
10	SOM175-e6&7				***	0.3
	SOM175-e4					***

Table 2.2

15 **A Percent amino acid identity between splice variants of SOM175 and human VEGF₁₆₅**

		VEGF ₁₆₅	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
20	VEGF ₁₆₅	***	31.4	42.3	33.5	40.6
	SOM175		***	74.7	73.7	99.1
	SOM175-e6			***	76.8	99.1
	SOM175-e6&7				***	99.1
	SOM175-e4					***

25

B Percent amino acid divergence between splice variants of SOM175 and human VEGF₁₆₅

5		VEGF ₁₆₅	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
	VEGF ₁₆₅	***	65.7	55.4	54.6	57.4
	SOM175		***	19.9	4.2	0.0
	SOM175-e6			***	0.0	0.0
10	SOM175-e6&7				***	0.0
	SOM175-e4					***

EXAMPLE 4

BIOASSAYS TO DETERMINE THE FUNCTION OF SOM175

Assays are conducted to evaluate whether SOM175 has similar activities to VEGF on endothelial cell function, angiogenesis and wound healing. Other assays are performed based on the results of receptor binding distribution studies.

Assays of endothelial cell function

Endothelial cell proliferation. Endothelial cell growth assays as described in Ferrara & Henzel (1989) and in Gospodarowicz *et al* (1989).

Vascular permeability assay. This assay, which utilises the Miles test in guinea pigs, will be performed as described in Miles & Miles (1952).

Cell adhesion assay. The influence of SOM175 on adhesion of polymorphs to endothelial cells is analysed.

Chemotaxis. This is performed using the standard Boyden chamber chemotaxis assay.

Plasminogen activator assay. Endothelial cells are tested for plasminogen activator and plasminogen activator inhibitor production upon addition of SOM175 (Pepper *et al* (1991)).

- 5 *Endothelial cell migration assay.* The ability of SOM175 to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano *et al* (1986).

Angiogenesis Assay

- SOM175 induction of an angiogenic response in chick chorioallantoic membrane is
10 evaluated as described in Leung *et al* (1989).

Possible neurotrophic actions of SOM175 are assessed using the following assays:

Neurite outgrowth assay and gene induction (PC12 cells)

- 15 PC12 cells (a pheochromocytoma cell line) respond to NGF and other neurotrophic factors by developing the characteristics of sympathetic neurons, including the induction of early and late genes and the extension of neurites. These cells are exposed to SOM175 and their response monitored (Drinkwater *et al* (1991); and Drinkwater *et al* (1993)).

Cultured neurons from the Peripheral Nervous System (PNS)

Primary cultures of the following PNS neurons are exposed to SOM175 and monitored for any response:

- 20
25
 - sensory neurons from neural crest and dorsal root ganglia
 - sympathetic neurons from sympathetic chain ganglia
 - placode derived sensory neurons from nodose ganglia
 - motoneurons from spinal cord

The assays are described in Suter *et al* (1992) and in Marinou *et al* (1992).

- 30 Where an *in vitro* response is observed, *in vivo* assays for properties such as uptake and retrograde transport are performed as described in Hendry *et al* (1992).

Nerve regeneration (PNS)

Where neurotrophic effects of SOM175 are observed, its possible role in the regeneration of axotomised sensory neurons, sympathetic neurons and motoneurons is analysed by the methods of Otto *et al* (1989); Yip *et al* (1984) and Hendry *et al* 5 (1976).

Actions of SOM175 on CNS neurons

The ability of SOM175 to promote survival of central nervous system neurons is analysed as described in Hagg *et al* (1992); Williams *et al* (1986); Hefti (1986) and 10 Kromer (1987).

Wound Healing

The ability of SOM175 to support wound healing are tested in the most clinically relevant model available, as described in Schilling *et al* (1959) and utilised by Hunt 15 *et al* (1967).

The Haemopoietic System

A variety of *in vitro* and *in vivo* assays on specific cell populations of the haemopoietic system are available and are outlined below:

20 Stem Cells

Murine

A variety of novel *in vitro* murine stem cell assays have been developed using FACS-purified cells:

25 (a) Repopulating Stem Cells

These are cells capable of repopulating the bone marrow of lethally irradiated mice, and have the Lin⁻, Rh^{hi}, Ly-6A/E⁺, c-kit⁺ phenotype. The test substance is tested on these cells either alone, or by co-incubation with multiple factors, followed by measurement of cellular proliferation by ³H thymidine incorporation.

30

(b) Late Stage Stem Cells

These are cells that have comparatively little bone marrow repopulating ability but can generate D13 CFU-S. These cells have the Lin⁻, Rh^{hi}, Ly-6A/E⁺, c-kit⁺ phenotype. The test substance is incubated with these cells for a period of time, injected into lethally irradiated recipients, and the number of D13 spleen colonies enumerated.

(c) Progenitor-Enriched Cells

These are cells that respond *in vitro* to single growth factors, and have the Lin⁻, Rh^{hi}, Ly-6A/E⁺, c-kit⁺ phenotype. This assay will show if SOM175 can act directly on haemopoietic progenitor cells. The test substance is incubated with these cells in agar cultures, and the number of colonies enumerated after 7-14 days.

15 Atherosclerosis

Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change in their phenotype from a contractile to a synthetic state. Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic modulations of smooth muscle cell. An *in vitro* assay that measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment is used to assess the effect of SOM175 on smooth muscle cells. The system uses a modified Rose chamber in which different cell types are seeded onto opposite coverslips.

25

Effects of SOM175 on bone

The ability of SOM175 to regulate proliferation of osteoblasts is assayed as described in Lowe *et al* (1991). Any effects on bone resorption are assayed as described in Lowe *et al* (1991). Effects on osteoblast migration and changes in intracellular molecules (e.g. cAMP accumulation, alkaline phosphatase levels) are analysed as described in Midy *et al* (1994).

30

Effects on skeletal muscle cells

Effects of SOM175 on proliferation of myoblasts and development of myotubes can be determined as described by Ewton *et al* (1980) and by Gospodarowicz *et al* (1976).

5

EXAMPLE 5

CLONING MURINE VEGF DNA

Isolation of cDNAs

Murine VRF (mVRF) clones were selected from a lambda Zap new born whole brain
10 cDNA library (Stratagene). Primary phage from high density filters (5×10^4
pfu/plate) were identified by hybridisation with a 682bp ^{32}P -labelled probe generated
by PCR from an hVRF cDNA (pSOM175) as described above. Hybridisation and
stringent washes of nylon membranes (Hybond-N) were carried out at 65°C under
conditions described by Church and Gilbert (1984). Positive plaques were picked,
15 purified and excised *in vivo* to produce bacterial colonies containing cDNA clones in
pBluescript SK-.

Isolation of genomic clones

Genomic clones were isolated from a mouse strain SV/129 library cloned in the
20 lambda Fix II vector (Stratagene). High density filters (5×10^4 pfu/filter) were
screened with a 563 bp ^{32}P -labelled probe generated by PCR amplification of the
nucleotide 233-798 region of the mVRF cDNA (see Figure 9). Positive clones were
plugged and re-screened with filters containing 400-800 pfu. Large scale phage
preparations were prepared using the QIAGEN lambda kit or by ZnCl_2 purification
25 (Santos, 1991).

Nucleotide sequencing and analysis

cDNAs were sequenced on both strands using a variety of vector-based and internal
primers with Applied Biosystems Incorporated (ABI) dye terminator sequencing kits
30 according to the manufacturer's specifications. Sequences were analysed on an ABI
Model 373A automated DNA sequencer. Peptide homology alignments were
performed using the program BESTFIT (GCG, Wisconsin).

Identification of intron/exon boundaries

Identification of exon boundaries and flanking regions was carried out using PCR with mouse genomic DNA or mVRF genomic lambda clones as templates. The primers used in PCR to identify introns were derived from the hVRF sequence and to
5 allow for potential human-mouse sequence mismatches annealing temperatures 5-10°C below the estimated T_m were used. All PCR products were sized by agarose gel electrophoresis and gel purified using QIAquick spin columns (Qiagen) and the intron/exon boundaries were sequenced directly from these products. In addition, some splice junctions were sequenced from subcloned genomic fragments of MVRF.
10 Intron/exon boundaries were identified by comparing cDNA and genomic DNA sequences.

Northern analysis

Total cellular RNA was prepared from a panel of fresh normal adult mouse tissues
15 (brain, kidney, liver, muscle) using the method of Chomczynski and Sacchi (1987). 20µg of total RNA were electrophoresed, transferred to a nylon membrane (Hybond N, Amersham) and hybridised under standard conditions (Church & Gilbert, 1984). Filters were washed at 65°C in 0.1xSSC (20xSSC is 3M NaCl/0.3M trisodium citrate), 0.1% SDS and exposed to X-ray film with intensifying screens at -70°C for
20 1-3 days.

Characterisation of mVRF cDNAs

Murine VRF homologues were isolated by screening a murine cDNA library with an hVRF cDNA clone. Five clones of sizes varying from 0.8-1.5 kb were recovered
25 and sequenced. The cDNA sequences were compiled to give a full length 1041 bp cDNA sequence covering the entire open reading frame (621 bp or 564 bp depending on the splice form, see below) and 3' UTR (379 bp), as well as 163 bp of the 5' UTR (Figure 9).

30 The predicted initiation codon matched the position of the start codon in hVRF. One other out of frame ATG was located at position -47 and two termination codons were observed upstream (positions -9 and -33, respectively) and in-frame with the putative

initiation codon.

The predicted N-terminal signal peptide of hVRF appears to be present in mVRF with 81% identity (17/21 amino acids). Peptide cleavage within mVRF is expected to occur after residue 21 (Figure 10). These data suggest that mature mVRF is secreted and could therefore conceivably function as a growth factor.

As with hVRF, two open reading frames (ORFs) were detected in cDNAs isolated by library screening. Four of five clones were found to be alternatively spliced and lacked a 101 bp fragment homologous to exon 6 of hVRF. The predicted peptide sequences of the two isoforms of mVRF were determined and aligned with the corresponding human isoforms (Figure 10).

The message encoding mVRF₁₈₆ contains a 621 bp ORF with coding sequences terminating at position +622, towards the end of exon 7 (Figure 9). The smaller message encoding mVRF₁₆₇ actually terminates downstream of the +622 TAG site due to a frame shift resulting from splicing out of the 101 bp exon 6 and the introduction of a stop codon (TGA) at position +666, near the beginning of exon 8 (Figure 9).

The mVRF₁₈₆ protein has strong homology to the amino and central portions of VEGF while the carboxyl end is completely divergent and is alanine rich. mVRF₁₆₇ possesses these similarities and also maintains homology to mVEGF right through to the C-terminus (Figure 11). The overall homology of mVRF₁₆₇ to hVRF₁₆₇ was 85% identity and 92% similarity, respectively (Figure 10). Likewise, homology between mVRF₁₆₇ and mVEGF (Breier *et al*, 1992) was 49% identity and 71% conservative amino acid substitution, respectively (Figure 11).

A canonical vertebrate polyadenylation signal (AATAAA) (Birnstiel *et al*, 1986) was not present in the mVRF cDNA, however, the closely matching sequence GATAAA is present at similar positions in both mouse and human VRF cDNAs (Figure 9). In contrast to hVRF, mVRF was found to contain an AC dinucleotide repeat at the

extreme 3' end of the 3' UTR (nucleotide positions 998 to 1011, Figure 9).

Polymorphism of this repeat region was observed between some of the mVRF cDNAs, with the number of dinucleotides varying from 7 to 11.

5 Genomic characterisation of mVRF

Intron/exon boundaries (Table 3) were mapped using primers which flanked sequences homologous to the corresponding hVRF boundaries. Introns I, III, IV and VI of mVRF (Table 3, Figure 12) were smaller than the hVRF intervening sequences. The complete genomic sequence was compiled from the 5' UTR of mVRF through to intron VI, the largest intervening region (2.2 kb), by sequencing amplified introns and cloned genomic portions of mVRF. There was only one major difference in genomic structure between mVRF and hVRF and that was the exon 7/intron VI boundary of mVRF was located 10bp further downstream in relation to the cDNA sequence, hence exon 7 in mVRF is 10bp longer than the corresponding exon in hVRF.

Exons 6 and 7 are contiguous in mVRF, as has been found to occur in the human homologue. The strong sequence homology between exon 6 of mVRF and hVRF (Figure 10) suggests that this sequence is not a retained intronic sequence but rather encodes a functional part of the VRF₁₈₆ isoform.

General intron/exon structure is conserved between the various members of the VEGF gene family (VEGF, PIGF, hVRF) and therefore it is not surprising that the overall genomic organisation of the mVRF gene is very similar to these genes (Figure 12).

Previous comparative mapping studies have shown that the region surrounding the human multiple endocrine neoplasia type 1 disease locus on chromosome 11q13 is syntenic with the proximal segment of mouse chromosome 19 (Rochelle *et al*, 1992). Since the inventors have mapped the hVRF gene to within 1kb of the human *MEN1* locus (see above) it is most likely that the murine VRF gene maps near the centromere of chromosome 19.

Expression studies of mVRF

Northern analysis of RNA from adult mouse tissues (muscle, heart, lung and liver) showed that expression appears to be ubiquitous and occurs primarily as a major band of approximately 1.3kb in size (Figure 14). This is somewhat different to the pattern observed for hVRF in which two major bands of 2.0 and 5.5 kb have been identified in all tissues examined. The 1.3 kb murine message presumably corresponds to the shorter of the human transcripts and the size variation thereof is most likely due to a difference in the length of the respective 5' UTRs.

EXAMPLE 6

EXPRESSION OF MURINE VEGF IN PRE- AND POST-NATAL MOUSE

Animals

Timed pregnant (n=4) and young adult (n=2) mice (C57 inbred strain, ALAB, Sweden) were sacrificed with carbon dioxide, and the relevant tissues were taken out and frozen on a chuck. Tissues were kept at -70°C until further use. Two gestational ages was used in this study; embryonic day 8 (E8), 14 and E17.

In situ hybridisation histochemistry

In situ hybridisation was performed as previously described (Dagerlind *et al*, 1992).

Briefly, transverse sections (14µm) were cut in a cryostat (Microm, Germany), thawed onto Probe-On slides (Fisher Scientific, USA) and stored in black sealed boxes at -70°C until used. The sequences of the synthetic 42-mer oligonucleotides complementary to mRNA encoding mVRF were

ACCACCACCTCCCTGGGCTGGCATGTGGCACGTGCATAAACG [SEQ ID NO:11] (complementary to nt 120-161) and

AGTTGTTTGACCACATTGCCCATGAGTTCCATGCTCAGAGGC [SEQ ID NO:12] (complementary to nt 162-203). To detect the two alternative splice forms oligonucleotide GATCCTGGGGCTGGAGTGGGATGGATGATGTCAGCTGG [SEQ ID NO:13] (complementary to nt xxx-xxx) and

GCGGGCAGAGGATCCTGGGGCTGTCTGGCCTCACAGCACT [SEQ ID NO:14] were used. The probes were labeled at the 3'-end with deoxyadenosine-alpha[thio]triphosphate [³⁵S] (NEN, USA) using terminal deoxynucleotidyl

transferase (IBI, USA) to a specific activity of $7-10 \times 10^8$ cpm/ μ g and hybridised to the sections without pretreatment for 16-18 h at 42°C. The hybridisation mixture contained: 50% v/v formamide, 4 x SSC (1 x SSC = 0.15M NaCl and 0.015M sodium-citrate), 1 x Denhardt's solution (0.02% each of polyvinyl-pyrrolidone, BSA and Ficoll), 1% v/v sarcosyl (N-lauroylsarcosine; Sigma), 0.02M phosphate buffer (pH 7.0), 10% w/v dextran sulfate (Pharmacia, Sweden), 250 μ g/ml yeast tRNA (Sigma), 500 μ g/ml sheared and heat denatured salmon sperm DNA (Sigma) and 200mM dithiothreitol (DTT; LKB, Sweden). In control sections, the specificity of both probes was checked by adding a 20-fold excess of unlabeled probe to the hybridisation mixture. In addition, adjacent sections were hybridised with a probe unrelated to this study which gave a different expression pattern. Following hybridisation the sections were washed several times in 1 x SSC at 55°C, dehydrated in ethanol and dipped in NTB2 nuclear track emulsion (Kodak, USA). After 3-5 weeks the sections were development in D-19 developer (Kodak, USA) and cover-slipped. In some cases, sections were exposed to an autoradiographic film (Beta-max autoradiography film Amersham Ltd, UK) prior to emulsion-dipping.

The four different probes gave identical hybridisation patterns in all tissues examined. Mouse VRF expression was detected already in the E8 embryo, in which positive signal was recorded over structures most likely corresponding to the neuronal tube. In sagittal sections of E14 mouse embryo the strongest hybridisation signal was present over heart and in the nervous system, especially cerebral cortex (Figure 14A). A low level of expression was present in all other tissues. At a later gestational age, E17, a high mVRF mRNA signal was confined to the heart and brown fat tissue in the back and around the neck (Figure 14B). Clearly positive hybridisation signals were present in the gray of the spinal cord and in the tongue (Figure 14B). Expression in the cerebral cortex was clearly reduced compared to day 14. The weak background expression seen in the E14 embryo in for example muscle, had decreased at this gestational age. A strong mVRF mRNA hybridisation signal was present solely over the heart and in the brown fat in the young adult mice (Figure 14C). The signal over the heart was evenly distributed over the entire ventricular wall, including the papillary muscles (Figure 14D). In sections of heart tissue hybridised with an

excess of cold probe, no specific labeling over background signal was recorded (Figure 14E).

Apart from the heart, mVRF mRNA signal was present over certain tissues on the
5 outside of the thoracic cage that morphologically resembled brown fat. This was
verified with sudan black counterstaining, which showed a strong staining in the
same areas (Figure 15A and 15B). In transverse sections of adult mouse spinal cord,
the mVRF probes gave a neuronal staining pattern over the gray matter (Figure 15C).
Counterstaining with toluidine (Figure 15D) showed that motoneurons in the ventral
10 horn (Figure 15C and 15D), interneurons (Figure 15C) in the deep part of the dorsal
horn and around the central canal where to a large extent positive for mVRF mRNA.

EXAMPLE 7

EFFECTS OF VEGF AND SOM175 PROTEINS ON CHICK

15 SENSORY NEURONS

The effects of VEGF and SOM175 proteins on embryonic day 8 chick sensory
neurons were determined using the method of Nurcombe *et al* (1992). The neuronal
assay was read at 48 hours using 2000 cells per assay well. The results were
obtained using ³H-thymidine counts. The percentage survival of neurons, neurite
20 outgrowth and average neurite length in μm were determined using NGF as positive
control and various concentrations of VEGF, VEGF in the presence of heparin and
VEGF in the presence of heparin and 5 μM , 5'-flurouracil (5FU). 5FU kills glial
cells.

25 The results are shown in Figure 16. The results show that VEGF is effective in
promoting neuronal survival but that this requires the presence of glial cells. Figure
17 shows the results of the effect of VEGF and SOM175 on three types of chick
glia. The glia tested were CNS glia, peripheral glia and CNS oligodendrocytes.
Heparin was used as 10 $\mu\text{g/ml}$ in all cultures and the assay was read at 24 hours.
30 Results were measured in ³H-thymidine counts using 2000 cells per well.

The results show that for chick central and peripheral neurons, astroglia were markedly stimulated to proliferate by SOM175 in the presence of heparin but that chick oligodendrocytes showed negligible increase in the rate of division.

5

EXAMPLE 8

EFFECTS OF SOM175 PROTEINS ON MOUSE PRIMARY AND CENTRAL NEURONS

The results in Example 7 show that VEGF isoform had an effect on chick primary and central neurons through the agency of the astroglial cells. Similar experiments were repeated in mouse cells.

Culture conditions

Neuronal and glial cells for all *in vitro* experiments were prepared and cultured according to the techniques described in "Methods in Neurosciences (Vol. 2): Cell Culture" Ed. P.M. Conn, Academic Press, San Diego, 1990, pp33-46 for astroglial cells, pp56-74 for oligodendroglial cells, and pp87-102 for central neurons.

Cells were plated onto 24-well culture clusters (Nunc) coated with poly-L-ornithine (0.1 mg/ml, 1h) at a density of 2,000 cells/well. After 48 hours in culture, neurons were counted in the wells under inverted phase light using well established techniques (Maruta *et al.* 1993) and glial cells assessed with [³H]thymidine uptake to monitor cell division rates as below. Heparin (10µg/ml, low molecular weight fraction, Sigma Chemical Corp.) was present at all times in the culture media except where noted. The neuronal cultures were supplemented with 5mM 5-fluoro-2-deoxyuridine (Sigma) to suppress background glial growth.

³H-Thymidine incorporation assay for glial cell proliferation

The cells were pulsed for 14h with ³H-thymidine (specific activity 103 µCi/ug) from a stock concentration of 0.1 mCi/ml in standard medium, giving a final incubating volume of 20 µl/well. The contents of the wells were harvested and absorbed onto nitrocellulose paper (Titertek, Flow). Remaining adherent cells were removed by

incubation with trypsin/versene (CSL Limited, Victoria, Australia) for 5 min. This procedure was carried out twice. The nitrocellulose discs were washed in a standard Titertek harvester (Flow) using first distilled water, and then methanol. The nitrocellulose discs were dried, scintillation fluid (containing 5% v/v Triton-X) added
5 and the discs counted on a scintillation counter.

Greatest activity was seen with preparations of SOM175 absent exon 6 (SOM Δ X6) on mouse astroglial cell cultures, where there was a significant stimulus to their proliferation when delivered in conjunction with heparin (Figure 16). Little stimulus
10 was given to the proliferation of oligodendroglial cells (Figure 17), and very little discernable potentiation of the survival response of isolated forebrain neurons (Figure 18). The standard deviation on all three graphs for each point was less than 8%.

The viability of neurons can be maintained by promoting glial cell proliferation.
15 Furthermore, SOM Δ X6 is a good inducer of astroglial proliferation and may be expressed in conjunction with the formation of astroglial endfeet on central nervous system endothelial cells.

Those skilled in the art will appreciate that the invention described herein is
20 susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

25

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TABLE 3
Splice junctions of the murine VRF gene

5	5' UTR*	Exon 1 >223bp	CCCAGgtacgtgcgt	Intron I	495bp
	ttccccacagGCCCC	Exon 2 43bp	GAAAGgtaataatag	Intron II	288bp
	ctgcccacagTGGTG	Exon 3 197bp	TGCAGgtaccagggc	Intron III	196bp
	ctgagcacagATCCT	Exon 4 74bp	TGCAGgtgccagccc	Intron IV	182bp
	ctcttttcagACCTA	Exon 5 36bp	GACAGattcttggtg	Intron V	191bp
10	ctcctcctagGGTTG	Exon 6 101bp		(no intron)	
	CCCACCTCCAGCCCCA	Exon 7 135bp	TGTAGgtaaggagtc	Intron VI	~2200bp
	cactccccagGTGCC	Exon 8 394bp	AGAGATGGAGACACT		

Uppercase and lowercase letters denote exonic and intronic sequences respectively.

15 * Indicates that the 5' end of exon 1 has not yet been determined.

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(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777

(B) TELEFAX: +61 3 9254 2770

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 649 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 17...589

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCGGGCCTCC GAAACC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC 49
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser
1 5 10

CTT GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA 97
Leu Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala
15 20 25

CCC ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC 145
Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe
30 35 40

ATG GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG 193
Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val
45 50 55

GAC ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA 241
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro
60 65 70 75

TCC TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC 289
Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly
80 85 90

CTG GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG	337
Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met	
95 100 105	
CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA	385
Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu	
110 115 120	
CAG CAC AAC AAA TGT GAA TGC AGA CCA AAG AAA GAT AGA GCA AGA CAA	433
Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln	
125 130 135	
GAA AAT CCC TGT GGG CCT TGC TCA GAG CGG AGA AAG CAT TTG TTT GTA	481
Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val	
140 145 150 155	
CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT	529
Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg	
160 165 170	
TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC	577
Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp	
175 180 185	
AAG CCG AGG CGG TGAGCCGGGC AGGAGGAAGG AGCCTCCCTC AGCGTTTCGG	629
Lys Pro Arg Arg	
190	
GAACCAGATC TCTCACCAGG	649

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 191 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu	
1 5 10 15	
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly	
20 25 30	
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln	
35 40 45	
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu	
50 55 60	

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130 135 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145 150 155 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165 170 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185 190

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1094 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG 47
Met Ser Pro Leu Leu Arg Arg Leu Leu Ala Ala Leu Leu Gln
1 5 10 15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC 95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
20 25 30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC 143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
35 40 45

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CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr 50 55 60	191
GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly 65 70 75	239
GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His 80 85 90 95	287
CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu 100 105 110	335
GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys 115 120 125	383
AAA AAG GAC AGT GCT GTG AAG CCA GAC AGG GCT GCC ACT CCC CAC CAC Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His 130 135 140	431
CGT CCC CAG CCC CGT TCT GTT CCG GGC TGG GAC TCT GCC CCC GGA GCA Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala 145 150 155	479
CCC TCC CCA GCT GAC ATC ACC CAT CCC ACT CCA GCC CCA GGC CCC TCT Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser 160 165 170 175	527
GCC CAC GCT GCA CCC AGC ACC ACC AGC GCC CTG ACC CCC GGA CCT GCC Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala 180 185 190	575
GCT GCC GCT GCC GAC GCC GCA GCT TCC TCC GTT GCC AAG GGC GGG GCT T Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala 195 200 205	624
AGAGCTCAAC CCAGACACCT GCAGGTGCCG GAAGCTGCGA AGGTGACACA TGGCTTTTCA	684
GACTCAGCAG GGTGACTTGC CTCAGAGGCT ATATCCCAGT GGGGGAACAA AGGGGAGCCT	744
GGTAAAAAAC AGCCAAGCCC CCAAGACCTC AGCCAGGCA GAAGCTGCTC TAGGACCTGG	804
GCCTCTCAGA GGGCTCTTCT GCCATCCCTT GTCTCCCTGA GGCCATCATC AAACAGGACA	864
GAGTTGGAAG AGGAGACTGG GAGGCAGCAA GAGGGGTCAC ATACCAGCTC AGGGGAGAAT	924
GGAGTACTGT CTCAGTTTCT AACCCTCTG TGCAAGTAAG CATCTTACAA CTGGCTCTTC	984
CTCCCTCAC TAAGAAGACC CAAACCTCTG CATAATGGGA TTTGGGCTTT GGTACAAGAA	1044
CTGTGACCCC CAACCCTGAT AAAAGAGATG GAAGGAAAAA AAAAAAAAAA	1094

(i) SEQUENCE CHARACTERISTICS:

(B) TYPE: amino acid

(ii) MOLECULE TYPE: protein

Met	Ser	Pro	Leu	Leu	Arg	Arg	Leu	Leu	Pro	Ala	Ala	Leu	Leu	Gln	Leu
1				5					10					15	
Ala	Pro	Ala	Gln	Ala	Pro	Val	Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	Gln
			20					25					30		
Arg	Lys	Val	Val	Ser	Trp	Ile	Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	Gln
		35					40					45			
Pro	Arg	Glu	Val	Val	Val	Pro	Leu	Thr	Val	Glu	Leu	Met	Gly	Thr	Val
	50					55					60				
Ala	Lys	Gln	Leu	Val	Pro	Ser	Cys	Val	Thr	Val	Gln	Arg	Cys	Gly	Gly
65					70					75					80
Cys	Cys	Pro	Asp	Asp	Gly	Leu	Glu	Cys	Val	Pro	Thr	Gly	Gln	His	Gln
				85					90					95	
Val	Arg	Met	Gln	Ile	Leu	Met	Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	Gly
			100					105					110		
Glu	Met	Ser	Leu	Glu	Glu	His	Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	Lys
		115					120					125			
Lys	Asp	Ser	Ala	Val	Lys	Pro	Asp	Arg	Ala	Ala	Thr	Pro	His	His	Arg
	130					135					140				
Pro	Gln	Pro	Arg	Ser	Val	Pro	Gly	Trp	Asp	Ser	Ala	Pro	Gly	Ala	Pro
145					150					155					160
Ser	Pro	Ala	Asp	Ile	Thr	His	Pro	Thr	Pro	Ala	Pro	Gly	Pro	Ser	Ala
				165					170					175	
His	Ala	Ala	Pro	Ser	Thr	Thr	Ser	Ala	Leu	Thr	Pro	Gly	Pro	Ala	Ala
			180					185					190		
Ala	Ala	Ala	Asp	Ala	Ala	Ala	Ser	Ser	Val	Ala	Lys	Gly	Gly	Ala	
			195				200					205			

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG      47
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
  1                      5                      10                      15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC      95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                20                      25                      30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC      143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
          35                      40                      45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC      191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
          50                      55                      60

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT      239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
          65                      70                      75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC      287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
          80                      85                      90                      95

CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG      335
Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu
                100                      105                      110

GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA      383
Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys
                115                      120                      125

AAA AAG GAC AGT GCT GTG AAG CCA GAT AGC CCC AGG CCC CTC TGC CCA      431
Lys Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro
                130                      135                      140

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CGC TGC ACC CAG CAC CAC CAG CGC CCT GAC CCC CGG ACC TGC CGC TGC 479
 Arg Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys
 145 150 155

CGC TGC CGA CGC CGC AGC TTC CTC CGT TCC CAA GGG CGG GGC TTA GAG 527
 Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu
 160 165 170 175

CTC AAC CCA GAC ACC TGC AGG TGC CGG AAG CTG CGA AGG TGACACATGG 576
 Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
 180 185

CTTTTCAGAC TCAGCAGGGT GACTTGCCCTC AGAGGCTATA TCCCAGTGGG GGAACAAAGG 636

GGAGCCTGGT AAAAAACAGC CAAGCCCCCA AGACCTCAGC CCAGGCAGAA GCTGCTCTAG 696

GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC CATCATCAAA 756

CAGCACAGAG TTGGAAGAGG AGACTGGGAG GCACCAAGAG GGGTCACATA CCAGCTCAGG 816

GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT CTTACAACCTC 876

GCTCTTCCTC CCCTCACTAA GAAGACCCAA ACCTCTGCAT AATGGGATTT GGCCTTTTCCT 936

ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAAA AAAAAAA 997

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 188 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
 20 25 30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
 35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
 50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
 65 70 75 80

(2) INFORMATION FOR SEQ ID NO:7:

(A) LENGTH: 858 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) **FEATURE:**

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG	47
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln	
1 5 10 15	
CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC	95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His	
20 25 30	
CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC	143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys	
35 40 45	
CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC	191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr	
50 55 60	

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CQC TGT GGT	239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly	
65 70 75	
GGC TGC TGC CCT GAC GAT GGC CTG GAU TCT GTG CCC ACT GGG CAG CAC	287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His	
80 85 90 95	
CAG GTC CGG ATG CAG ATC CTC ATG ATC CCG TAC CCG AGC AGT CAG CTG	335
Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu	
100 105 110	
GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA	383
Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys	
115 120 125	
AAA AAG GAC AGT CCT GTG AAG CCA GAT AGG TGC CGG AAG CTG CGA AGC	431
Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Cys Arg Lys Leu Arg Arg	
130 135 140	
TGACACATGG CTTTTCAGAC TCAGCAGGGT GACTTGCCTC AGAGGCTATA TCCAGTGGG	491
GGAAACAAAGG GGAGCCTGGT AAAAAACAGC CAAGCCCCCA AGACCTCAGC CCAGGCCAAG	551
GCTGCTCTAG GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC	611
CATCATCAAA CAGGACAGAG TTGGAAGAGG AGACTGGGAG GCAGCAAGAG GGCTCACATA	671
CCAGCTCAGG GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT	731
CTTACAACTG GCTCTTCCTC CCCTCACTAA GAAGACCCAA ACCTCTGCAT AATGGGATTT	791
GGGCTTTGGT ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAA	851
AAAAAAA	858

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 143 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1             5             10             15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
          20             25             30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
          35             40             45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
          50             55             60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
          65             70             75             80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
          85             90             95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
          100            105            110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
          115            120            125

Lys Asp Ser Ala Val Lys Pro Asp Arg Cys Arg Lys Leu Arg Arg
          130            135            140

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(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 910 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 3..305

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG      47
Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln
  1                      5                      10                      15

CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC      95
Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His
                20                      25                      30

CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC      143
Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys
                35                      40                      45

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC      191
Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr
                50                      55                      60

GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT      239
Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
                65                      70                      75

GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC      287
Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
                80                      85                      90                      95

CAA GTC CGG ATG CAG ACC TAAAAAAAAG GACAGTGCTG TGAAGCCAGA      335
Gln Val Arg Met Gln Thr
                100

CAGGGCTGCC ACTCCCCACC ACCGTCCCCA GCCCGTTCT GTTCCGGGCT GGGACTCTGC      395

CCCCGGAGCA CCCTCCCCAG CTGACATCAC CCATCCCACT CCAGCCCCAG GCCCCTCTGC      455

CCACGCTGCA CCCAGCACCA CCAGCGCCCT GACCCCCGGA CCTGCCGCTG CCGCTGCCGA      515

CGCCGCAGCT TCCTCCGTTG CCAAGGGCGG GGCTTAGAGC TCAACCCAGA CACCTGCAGG      575

TGCCGGAAGC TGCGAAGGTG ACACATGGCT TTTCAGACTC AGCAGGGTGA CTTGCCTCAG      635

AGGCTATATC CAGTGGGGA ACAAAGAGGA GCCTGGTAAA AAACAGCCAA GCCCCAAGA      695

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CCTCAGCCCA GGCAGAAGCT GCTCTAGGAC CTGGGCCTCT CAGAGGGGCTC TTCTGCCATC      755
CCTTGTCTCC CTGAGGCCAT CATCAAACAG GACAGAGTTG GAAGAGGAGA CTGGGAGGCA      815
GCAAGAGGGG TCACATACCA GCTCAGGGGA GAATGGAGTA CTGTCTCAGT TTCTAACCAC      875
TCTGTGCAAG TAAGCATCTT ACAACTGGCT CTTCC                                   910

```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1             5             10             15
Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
          20             25             30
Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
          35             40             45
Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
          50             55             60
Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
          65             70             75             80
Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
          85             90             95
Val Arg Met Gln Thr
          100

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(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACCACCACCT CCCTGGGCTG GCATGTGGCA CGTGCATAAA CG

42

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGTTGTTTGA CCACATTGCC CATGAGTTCC ATGCTCAGAG GC

42

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCCTGGGG CTGGAGTGGG ATGGATGATG TCAGCTGG

38

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(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCGGGCAGAG GATCCTGGGG CTGTCTGGCC TCACAGCACT

40

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CLAIMS:

1. A biologically isolated proteinaceous molecule having the following characteristics:
 - (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).
2. A proteinaceous molecule according to claim 1 wherein the molecule exhibits at least one of the following properties:
 - (i) an ability to induce vascular endothelial cells;
 - (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
 - (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.
3. A proteinaceous molecule according to claim 1 or 2 wherein said molecule has the capacity to induce astroglial proliferation.
4. A proteinaceous molecule according to claim 1 wherein said molecule is of human origin.
5. A proteinaceous molecule according to claim 1 wherein said molecule is of non-human origin.
6. A proteinaceous molecule according to claim 5 wherein said molecule is of livestock animal, companion animal, laboratory test animal, avian, fish or reptilian origin.
7. A proteinaceous molecule according to claim 5 wherein said molecule is encoded by a gene located at chromosome 11q13.

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8. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.
9. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 40%.
10. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 60-70%.
11. A proteinaceous molecule according to claim 1 comprising a sequence of amino acids as set forth in SEQ ID NO:4 or a part, fragment, derivative or analogue thereof.
12. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:6 or a part, fragment, derivative or analogue thereof.
13. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:8 or a part, fragment, derivative or analogue thereof.
14. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:10 or a part, fragment, derivative or analogue thereof.
15. A recombinant molecule having the following characteristics:
 - (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.

16. A recombinant molecule having the following characteristics:
- (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.
17. A recombinant molecule having the following characteristics:
- (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.
18. A recombinant molecule having the following characteristics:
- (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one biological property in common with VEGF.
19. A recombinant molecule according to claim 15 or 16 or 17 or 18 having at least one of the following properties:
- (a) an ability to induce vascular endothelial cells;
 - (b) an ability to interact with *flt1/flki* family of receptors;
 - (c) an ability to induce cell migration, cell survival and/or increase intracellular levels of alkaline phosphatase.
20. A recombinant molecule according to claim 15 or 16 or 17 or 18 having the capacity to induce astroglial proliferation.

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21. A recombinant molecule according to claim 20 wherein the molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6.
22. A peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a derivative or chemical equivalent thereof.
23. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:6 or a chemical equivalent thereof.
24. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:8 or a chemical equivalent thereof.
25. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:10 or a chemical equivalent thereof.
26. A nucleic acid molecule comprising a sequence of nucleotides or complementary to a sequence encoding a proteinaceous molecule having the following characteristics:
- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).
27. A nucleic acid molecule according to claim 26 wherein the proteinaceous molecule exhibits at least one of the following properties:
- (i) an ability to induce vascular endothelial cells;
 - (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
 - (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.
28. A nucleic acid molecule according to claim 27 wherein the proteinaceous molecule has the capacity to induce astroglial proliferation.

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29. A nucleic acid molecule according to claim 28 wherein said molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:6.
30. A nucleic acid molecule according to claim 1 wherein said molecule is of human origin.
31. A nucleic acid molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.
32. A nucleic acid molecule according to claim 26 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.
33. A nucleic acid molecule according to claim 26 encoding a murine homologue of human VEGF and comprising a nucleotide sequence substantially as set forth in Figure 9.
34. A pharmaceutical composition comprising a proteinaceous molecule according to claim 1 or 2 or 3 or 11 and one or more pharmaceutically acceptable carriers and/or diluents.
35. A method for preparing a recombinant molecule having the following characteristics:
- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
 - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),
- said method comprising expressing a nucleic acid molecule encoding said recombinant

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molecule by a suitable host grown under conditions effective to synthesise said recombinant molecule and then isolating said molecule.

36. A method according to claim 35 wherein the nucleic acid molecule comprises a sequence of nucleotides as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or is capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.

37. A method of inducing astroglial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

38. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

39. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

40. A method of promoting neuronal survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

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- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

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ABSTRACT

The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.

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<i>2/52</i> <i>Fig.1(i)</i>	<i>3/52</i> <i>Fig.1(ii)</i>
<i>4/52</i> <i>Fig.1(iii)</i>	<i>5/52</i> <i>Fig.1(iv)</i>

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1	TCGGCCTCC	GAAACC	ATG	AAC	TTT	CTG		
			Met	Asn	Phe	Leu		
			1					
50	CTT	GCC	TTG	CTG	CTC	TAC	CTC	CAC
	Leu	Ala	Leu	Leu	Leu	Tyr	Leu	His
			15					
98	CCC	ATG	GCA	GAA	GGA	GGA	GGG	CAG
	Pro	Met	Ala	Glu	Gly	Gly	Gly	Gln
			30					35
146	ATG	GAT	GTC	TAT	CAG	CGC	AGC	TAC
	Met	Asp	Val	Tyr	Gln	Arg	Ser	Tyr
		45					50	
194	GAC	ATC	TTC	CAG	GAG	TAC	CCT	GAT
	Asp	Ile	Phe	Gln	Glu	Tyr	Pro	Asp
	60					65		
242	TCC	TGT	GTG	CCC	CTG	ATG	CGA	TGC
	Ser	Cys	Val	Pro	Leu	Met	Arg	Cys
					80			
290	CTC	GAG	TGT	GTG	CCC	ACT	GAG	GAG
	Leu	Glu	Cys	Val	Pro	Thr	Glu	Glu
				95				
338	CGG	ATC	AAA	CCT	CAC	CAA	GGC	CAG
	Arg	Ily	Lys	Pro	His	Gln	Gly	Gln
			110					115

Fig.1(i)

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CTG	TCT	TGG	GTG	CAT	TGG	AGC		49
Leu	Ser	Trp	Val	His	Trp	Ser		
5					10			
CAT	GCC	AAG	TGG	TCC	CAG	GCT	GCA	97
His	Ala	Lys	Trp	Ser	Gln	Ala	Ala	
20					25			
AAT	CAT	CAC	GAA	GTG	GTG	AAG	TTC	145
Asn	His	His	Glu	Val	Val	Lys	Phe	
			40					
TGC	CAT	CCA	ATC	GAG	ACC	CTG	GTG	193
Cys	His	Pro	Ile	Glu	Thr	Leu	Val	
			55					
GAG	ATC	GAG	TAC	ATC	TTC	AAG	CCA	241
Glu	Ile	Glu	Tyr	Ile	Phe	Lys	Pro	
		70					75	
GGG	GGC	TGC	TGC	AAT	GAC	GAG	GGC	289
Gly	Gly	Cys	Cys	Asn	Asp	Glu	Gly	
	85					90		
TCC	AAC	ATC	ACC	ATG	CAG	ATT	ATG	337
Ser	Asn	Ile	Thr	Met	Gln	Ile	Met	
100					105			
CAC	ATA	GGA	GAG	ATG	AGC	TTC	CTA	385
His	Ile	Gly	Glu	Met	Ser	Phe	Leu	
			120					

Fig.1(iii)

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386	CAG	CAC	AAC	AAA	TGT	GAA	TGC	AGA
	Gln	His	Asn	Lys	Cys	Glu	Cys	Arg
		125					130	
434	GAA	AAT	CCC	TGT	GGG	CCT	TGC	TCA
	Glu	Asn	Pro	Cys	Gly	Pro	Cys	Ser
	140					145		
482	CAA	GAT	CCG	CAG	ACG	TGT	AAA	TGT
	Gln	Asp	Pro	Gln	Thr	Cys	Lys	Cys
					160			
530	TGC	AAG	GCG	AGG	CAG	CTT	GAG	TTA
	Cys	Lys	Ala	Arg	Gln	Leu	Glu	Leu
				175				
578	AAG	CCG	AGG	CGG	TGAGCCGGGC	AGGAG		
	Lys	Pro	Arg	Arg				
			190					
630	GAACCAGATC	TCTCACCAGG						

Fig.1(iii)

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CCA	AAG	AAA	GAT	AGA	GCA	AGA	CAA	433
Pro	Lys	Lys	Asp	Arg	Ala	Arg	Gln	
			135					
GAG	CGG	AGA	AAG	CAT	TTG	TTT	GTA	481
Glu	Arg	Arg	Lys	His	Leu	Phe	Val	
		150					155	
TCC	TGC	AAA	AAC	ACA	GAC	TCG	CGT	529
Ser	Cys	Lys	Asn	Thr	Asp	Ser	Arg	
	165					170		
AAC	GAA	CGT	ACT	TGC	AGA	TGT	GAC	577
Asn	Glu	Arg	Thr	Cys	Arg	Cys	Asp	
180					185			
GAAGG	AGCCTCCCTC	AGCGTTTCGG						629
								649

Fig.1(iv)

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<p>7/52</p> <p><i>Fig.2(i)</i></p>	<p>8/52</p> <p><i>Fig.2(ii)</i></p>
<p>9/52</p> <p><i>Fig 2(iii)</i></p>	<p>10/52</p> <p><i>Fig 2(iv)</i></p>
<p>11/52</p> <p><i>Fig 2(v)</i></p>	<p>12/52</p> <p><i>Fig 2(vi)</i></p>

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1	CC	ATG	AGC	CCT	CTG	CTC	CGC	CGC	
	Met	Ser	Pro	Leu	Leu	Arg	Arg		
	1				5				
48	CTG	GCC	CCC	GCC	CAG	GCC	CCT	GTC	
	Leu	Ala	Pro	Ala	Gln	Ala	Pro	Val	
					20				
96	CAG	AGG	AAA	GTG	GTG	TCA	TGG	ATA	
	Gln	Arg	Lys	Val	Val	Ser	Trp	Ile	
				35					
144	CAG	CCC	CGG	GAG	GTG	GTG	GTG	CCC	
	Gln	Pro	Arg	Glu	Val	Val	Val	Pro	
			50					55	
192	GTG	GCC	AAA	CAG	CTG	GTG	CCC	AGC	
	Val	Ala	Lys	Gln	Leu	Val	Pro	Ser	
		65					70		
240	GGC	TGC	TGC	CCT	GAC	GAT	GGC	CTG	
	Gly	Cys	Cys	Pro	Asp	Asp	Gly	Leu	
	80					85			
288	CAA	GTC	CGG	ATG	CAG	ATC	CTC	ATG	
	Gln	Val	Arg	Met	Gln	Ile	Leu	Met	
				100					
336	GGG	GAG	ATG	TCC	CTG	GAA	GAA	CAC	
	Gly	Glu	Met	Ser	Leu	Glu	Glu	His	
				115					

Fig.2(i)

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CTG	CTG	CTC	GCC	GCA	CTC	CTG	CAG	47
Leu	Leu	Leu	Ala	Ala	Leu	Leu	Gln	
		10					15	
TCC	CAG	CCT	GAT	GCC	CCT	GGC	CAC	95
Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	
	25					30		
GAT	GTG	TAT	ACT	CGC	GCT	ACC	TGC	143
Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	
40					45			
TTG	ACT	GTG	GAG	CTC	ATG	GGC	ACC	191
Leu	Thr	Val	Glu	Leu	Met	Gly	Thr	
				60				
TGC	GTG	ACT	GTG	CAG	CGC	TGT	GGT	239
Cys	Val	Thr	Val	Gln	Arg	Cys	Gly	
			75					
GAG	TGT	GTG	CCC	ACT	GGG	CAG	CAC	287
Glu	Cys	Val	Pro	Thr	Gly	Gln	His	
		90					95	
ATC	CGG	TAC	CCG	AGC	AGT	CAG	CTG	335
Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	
	105					110		
AGC	CAG	TGT	GAA	TGC	AGA	CCT	AAA	383
Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	
120					125			

Fig. 2(iii)

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384	AAA	AAG	GAC	AGT	GCT	GTG	AAG	CCA
	Lys	Lys	Asp	Ser	Ala	Val	Lys	Pro
			130					135
432	CGT	CCC	CAG	CCC	CGT	TCT	GTT	CCG
	Arg	Pro	Gln	Pro	Arg	Ser	Val	Pro
		145					150	
480	CCC	TCC	CCA	GCT	GAC	ATC	ACC	CAT
	Pro	Ser	Pro	Ala	Asp	Ile	Thr	His
	160					165		
528	GCC	CAC	GCT	GCA	CCC	AGC	ACC	ACC
	Ala	His	Ala	Ala	Pro	Ser	Thr	Thr
					180			
576	GCT	GCC	GCT	GCC	GAC	GCC	GCA	GCT
	Ala	Ala	Ala	Ala	Asp	Ala	Ala	Ala
					195			

Fig. 2(iii)

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GAC	AGG	GCT	GCC	ACT	CCC	CAC	CAC	431
Asp	Arg	Ala	Ala	Thr	Pro	His	His	
				140				
GGC	TGG	GAC	TCT	GCC	CCC	GGA	GCA	479
Gly	Trp	Asp	Ser	Ala	Pro	Gly	Ala	
			155					
CCC	ACT	CCA	GCC	CCA	GGC	CCC	TCT	527
Pro	Thr	Pro	Ala	Pro	Gly	Pro	Ser	
		170					175	
AGC	GCC	CTG	ACC	CCC	GGA	CCT	GCC	575
Ser	Ala	Leu	Thr	Pro	Gly	Pro	Ala	
	185					190		
TCC	TCC	GTT	GCC	AAG	GGC	GGG	GCT	T 624
Ser	Ser	Val	Ala	Lys	Gly	Gly	Ala	
200					205			

Fig. 2(iv)

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625	AGAGCTCAAC	CCAGACACCT	GCAGGTGCCG
685	GACTCAGCAG	GGTGACTTGC	CTCAGAGGCT
745	GGTAAAAAAC	AGCCAAGCCC	CCAAGACCTC
805	GCCTCTCAGA	GGGCTCTTCT	GCCATCCCTT
865	GAGTTGGAAG	AGGAGACTGG	GAGGCAGCAA
825	GGAGTACTGT	CTCAGTTTCT	AACCACTCTG
985	CTCCCCTCAC	TAAGAAGACC	CAAACCTCTG
1045	CTGTGACCCC	CAACCCTGAT	AAAAGAGATG

Fig.2(v)

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GAAGCTGCGA	AGGTGACACA	TGGCTTTTCA	684
ATATCCCAGT	GGGGGAACAA	AGGGGAGCCT	744
AGCCCAGGCA	GAAGCTGCTC	TAGGACCTGG	804
GTCTCCCTGA	GGCCATCATC	AAACAGGACA	864
GAGGGGTCAC	ATACCAGCTC	AGGGGAGAAT	924
TGCAAGTAAG	CATCTTACAA	CTGGCTCTTC	984
CATAATGGGA	TTTGGGCTTT	GGTACAAGAA	1044
GAAGGAAAAA	AAAAAAAAAA		1094

Fig.2(vi)

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<p>14/52</p> <p><i>Fig. 3(i)</i></p>	<p>15/52</p> <p><i>Fig. 3(ii)</i></p>
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Parameter	Value	Unit
Initial temperature	25.0	°C
Final temperature	25.0	°C
Initial pressure	1.013	bar
Final pressure	1.013	bar
Initial volume	0.001	m³
Final volume	0.001	m³
Initial mass	0.001	kg
Final mass	0.001	kg
Initial density	1000	kg/m³
Final density	1000	kg/m³
Initial viscosity	0.001	Pa·s
Final viscosity	0.001	Pa·s
Initial thermal conductivity	0.6	W/m·K
Final thermal conductivity	0.6	W/m·K
Initial specific heat capacity	4182	J/kg·K
Final specific heat capacity	4182	J/kg·K
Initial enthalpy	4182	J/kg
Final enthalpy	4182	J/kg
Initial entropy	1.306	J/kg·K
Final entropy	1.306	J/kg·K
Initial internal energy	1674	J/kg
Final internal energy	1674	J/kg
Initial Gibbs free energy	-2371	J/kg
Final Gibbs free energy	-2371	J/kg
Initial Helmholtz free energy	-2371	J/kg
Final Helmholtz free energy	-2371	J/kg
Initial chemical potential	-2371	J/kg
Final chemical potential	-2371	J/kg
Initial activity	1.0	
Final activity	1.0	
Initial fugacity	1.013	bar
Final fugacity	1.013	bar
Initial vapor pressure	0.003	bar
Final vapor pressure	0.003	bar
Initial saturation pressure	0.003	bar
Final saturation pressure	0.003	bar
Initial critical pressure	218.1	bar
Final critical pressure	218.1	bar
Initial critical temperature	373.9	°C
Final critical temperature	373.9	°C
Initial critical density	322	kg/m³
Final critical density	322	kg/m³
Initial critical viscosity	0.001	Pa·s
Final critical viscosity	0.001	Pa·s
Initial critical thermal conductivity	0.6	W/m·K
Final critical thermal conductivity	0.6	W/m·K
Initial critical specific heat capacity	4182	J/kg·K
Final critical specific heat capacity	4182	J/kg·K
Initial critical enthalpy	4182	J/kg
Final critical enthalpy	4182	J/kg
Initial critical entropy	1.306	J/kg·K
Final critical entropy	1.306	J/kg·K
Initial critical internal energy	1674	J/kg
Final critical internal energy	1674	J/kg
Initial critical Gibbs free energy	-2371	J/kg
Final critical Gibbs free energy	-2371	J/kg
Initial critical Helmholtz free energy	-2371	J/kg
Final critical Helmholtz free energy	-2371	J/kg
Initial critical chemical potential	-2371	J/kg
Final critical chemical potential	-2371	J/kg
Initial critical activity	1.0	
Final critical activity	1.0	
Initial critical fugacity	1.013	bar
Final critical fugacity	1.013	bar
Initial critical vapor pressure	0.003	bar
Final critical vapor pressure	0.003	bar
Initial critical saturation pressure	0.003	bar
Final critical saturation pressure	0.003	bar
Initial critical critical pressure	218.1	bar
Final critical critical pressure	218.1	bar
Initial critical critical temperature	373.9	°C
Final critical critical temperature	373.9	°C
Initial critical critical density	322	kg/m³
Final critical critical density	322	kg/m³
Initial critical critical viscosity	0.001	Pa·s
Final critical critical viscosity	0.001	Pa·s
Initial critical critical thermal conductivity	0.6	W/m·K
Final critical critical thermal conductivity	0.6	W/m·K
Initial critical critical specific heat capacity	4182	J/kg·K
Final critical critical specific heat capacity	4182	J/kg·K
Initial critical critical enthalpy	4182	J/kg
Final critical critical enthalpy	4182	J/kg
Initial critical critical entropy	1.306	J/kg·K
Final critical critical entropy	1.306	J/kg·K
Initial critical critical internal energy	1674	J/kg
Final critical critical internal energy	1674	J/kg
Initial critical critical Gibbs free energy	-2371	J/kg
Final critical critical Gibbs free energy	-2371	J/kg
Initial critical critical Helmholtz free energy	-2371	J/kg
Final critical critical Helmholtz free energy	-2371	J/kg
Initial critical critical chemical potential	-2371	J/kg
Final critical critical chemical potential	-2371	J/kg
Initial critical critical activity	1.0	
Final critical critical activity	1.0	
Initial critical critical fugacity	1.013	bar
Final critical critical fugacity	1.013	bar
Initial critical critical vapor pressure	0.003	bar
Final critical critical vapor pressure	0.003	bar
Initial critical critical saturation pressure	0.003	bar
Final critical critical saturation pressure	0.003	bar
Initial critical critical critical pressure	218.1	bar
Final critical critical critical pressure	218.1	bar
Initial critical critical critical temperature	373.9	°C
Final critical critical critical temperature	373.9	°C
Initial critical critical critical density	322	kg/m³
Final critical critical critical density	322	kg/m³
Initial critical critical critical viscosity	0.001	Pa·s
Final critical critical critical viscosity	0.001	Pa·s
Initial critical critical critical thermal conductivity	0.6	W/m·K
Final critical critical critical thermal conductivity	0.6	W/m·K
Initial critical critical critical specific heat capacity	4182	J/kg·K
Final critical critical critical specific heat capacity	4182	J/kg·K
Initial critical critical critical enthalpy	4182	J/kg
Final critical critical critical enthalpy	4182	J/kg
Initial critical critical critical entropy	1.306	J/kg·K
Final		

SCORE = 181 (92.4 BITS), EXPECT = 6.4e-20,
IDENTITIES = 33/75 (44%), POSITIVES = 48/75

```

QUERY:      91  PTGQHQVRMQILMIR 105
              PT + + MQI+ I+
SBJCT:      96  PTEESNITMOIMRIK 110

```

SCORE = 76 (38.8 BITS), EXPECT = 0.0011,
IDENTITIES = 12/19 (63%), POSITIVES = 16/19

```

QUERY:      110  QLGEMSLEEHSQCECRPKK  128
              ++GEMS  +H+  CECRPKK
SBJCT:      116  HIGEMSFLOHNKCECRPKK  134

```

SCORE = 72 (36.8 BITS), EXPECT = 0.0046,
IDENTITIES = 14/21 (66%), POSITIVES = 15/21

```

QUERY:      202  RCQGRGLELNPDTCRCKRLRR  222
              RC +R LELN   TCRC  K  RR
SBJCT:      195  RCKAROLELNERTCRCDKPRR  215

```

SCORE = 46 (23.5 BITS), EXPECT = 47.,
IDENTITIES = 6/10 (60%), POSITIVES = 9/10

```

QUERY:      187  DPRTCRCRCR  196
              DP+TC+C  C+
SBJCT:      181  DPQTCKCSCK  190

```

SUBSTITUTE SHEET (RULE 26)

Fig.3 (i)

15/52

GROWTH FACTOR PRECURSOR (VEGF)

$$P = 6.4e-20$$

(64%)

MGTVAKQLVPSCVTVQRCGGCCPDDGLECV 90
 + PSCV + RCGGCC D+GLECV
 PDEIEYIFKPSCVPLMRCGGCCNDEGLECV 95

$$\text{POISSON } P(2) = 9.1e-12$$

(84%)

$$\text{POISSON } P(3) = 3.6e-18$$

(71%)

$$\text{POISSON } P(4) = 7.3e-10$$

(90%)

Fig. 3(i)

16/52

<p>17/52</p> <p>Fig.4(i)</p>	<p>18/52</p> <p>Fig.4(ii)</p>
<p>19/52</p> <p>Fig.4(iii)</p>	<p>20/52</p> <p>Fig.4(iv)</p>

17/52

Gap Weight:3.00 Average Match:1.000
 Length Weight:0.100 Average Mismatch:-0.900
 Quality:100.9 Length:739
 Ratio:0.175 Gaps:30
 Percent Percent
 Similarity:69.703 Identity:69.703

28	ATGAGCCCTCTGCTCCGCCGCCTGC
17	ATGAACTTTCTGCT.....GTCT..
68	TGCAGCTGGCCCCCGCCCAGGCCCC
57	TGCTGCTCTACCTCCACCATGCCAA
118	CACCAGAGGA.....
106	AGAAGGAGGAGGGCAGAATCATCAC
140	GTGTATACTCGC.GCTACCTGCCAG
152	GTCTATCAGCGCAGCTA.CTGCCAT
194	T....GA.....CTGTGGAGCTCAT
201	TCCAGGAGTACCCTGATGAGATCGA
235	CCCAGCTGCGTGACTGTGCAGCGCT
239	CCATCCTGTGTGCCCCTGATGCGAT
285	CCTGGAGTGTGTGCCCCACTGGGCAG
289	CCTGGAGTGTGTGCCCCACTGAGGAG

Fig.4(i)

TGCTCGCCGCACT.....CC	67
...TGGGTGCATTGGAGCCTTGCCT	56
TGTCTCCCAGCCTGATGCCCCCTGGC	117
GTGGTCCCAGGCTGCA.CCCATGGC	105
.AAGTGGTG....TCATGGATAGAT	147
GAAGTGGTGAAGTTCATG....GAT	151
CCCCGGGAG...GTGGTGGTGCCCT	193
CCAATCGAGACCCTGGTGGACATCT	200
GGGCACCGTGGCCAAACAGCTGGTG	234
GTACATCTT...CAA.....G	238
GTGGTGGCTGCTGCCCTGACGATGG	284
GCGGGGGCTGCTGCAATGACGAGGG	288
CACCAAGTCCGGATGCAGAT.....	329
TCCAACATCACCATGCAGATTATGC	338

Fig.4(ii)
SUBSTITUTE SHEET (RULE 26)

19/52

330CCTCATGATCCGGTACC
339	GGATCAAACCTCA.....C
369	GTCCCTGGAAGAACACAGCCAGTGT
376	GAGCTTCCTACAGCACAAATGT
419	GTGCTGTGAAGCCAGACAGGGCTGC
423	G.....AGCAAGACAAG.....
469	CGTTCTGTTCCGGGCTGGGACTCTG
443	...TGTGGGCCTTGCTCAGA.....
519	CATCACCCATCCCCTCCAGCCCCA
468
569	GC.....ACCACCAGCGCCC
469	GCATTTGTTTGTACAA.....
609	TGCCGACGCCGCAGCTTCCTCCGTT
509	TG.CAAAAACACAGACTC..GCGTT
657	AACCCAGACACCTGCAGGTGCCGGA
554	AACGAACGTACTTGCAGATGTGACA

Fig.4(iii)

663020-156460

20/52

CGAGCAGTCAGC . . . TGGGGGAGAT	368
CAAG . . GCCAGCACATAGGAGAGAT	375
GAATGCAGACCTAAAAAAAGGACA	418
GAATGCAGACC . . . AAAGAAAGATA	422
CACTCCCCACCACCGTCCCCAGCCC	468
. AAAATCCC	442
CCCCCGGAGCACCTCCCCAGCTGA	518
. . . GCGGAGAA	467
GGCCCCTCTGCCCCACGCTGCACCCA	568
. A	468
TGACCCCCGGACCTGCCGCTGCCGC	608
. GATCCGCAGACGTGTAAATGTTCC	508
GCCAAGGGCGGGGC . . TTAGAGCTC	656
GC . . AAGGCGAGGCAGCTTGAGTTA	553
AGCTGCGAAGGTGA	695
AGCCGAGGCGGTGA	592

Fig.4(iv)

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22/52 <i>Fig.5(i)</i>	23/52 <i>Fig.5(ii)</i>	24/52 <i>Fig.5(iii)</i>
25/52 <i>Fig.5(iv)</i>	26/52 <i>Fig.5(v)</i>	27/52 <i>Fig.5(vi)</i>

22/52

165SOMSQ.MSF.msf MSF:687

Type: D Tuesday, June 20, 1995

Check:3140

1

VEGF165	ATGAACTTTCTGCTGTCTTGGGTG
SOM175	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e6	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e6&7	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e4	ATGAGCCCTCTGCTCCGCCGCCTG

81

VEGF165	CACCCATGGCAGAAGGAGGAGGGC
SOM175	TGCCCCTGGCCACCAGAGGAAAGT
SOM175-e6	TGCCCCTGGCCACCAGAGGAAAGT
SOM175-e6&7	TGCCCCTGGCCACCAGAGGAAAGT
SOM175-e4	TGCCCCTGGCCACCAGAGGAAAGT

161

VEGF165	CCAATCGAGACCCTGGTGGACATC
SOM175	GTGGTGGTGGCCCTTGACTG.TGGA
SOM175-e6	GTGGTGGTGGCCCTTGACTG.TGGA
SOM175-e6&7	GTGGTGGTGGCCCTTGACTG.TGGA
SOM175-e4	GTGGTGGTGGCCCTTGACTG.TGGA

241

VEGF165	GATGCGATGCGGGGGCTGCTGCAA
SOM175	GCAGCGCTGTGGTGGCTGCTGCCC
SOM175-e6	GCAGCGCTGTGGTGGCTGCTGCCC
SOM175-e6&7	GCAGCGCTGTGGTGGCTGCTGCCC
SOM175-e4	GCAGCGCTGTGGTGGCTGCTGCCC

Fig.5(i)

655643-7560160

23/52

CATTGGAGCCTTGCCCTTGCTGCTCTACC
CTGCTCGCCGCACTCCTGCAGCTGGCCC
CTGCTCGCCGCACTCCTGCAGCTGGCCC
CTGCTCGCCGCACTCCTGCAGCTGGCCC
CTGCTCGCCGCACTCCTGCAGCTGGCCC

AGAATCATCACGAAGTGGTGAAGTTCAT
GGTGTCATGGATAGATGTGTATACTCGC
GGTGTCATGGATAGATGTGTATACTCGC
GGTGTCATGGATAGATGTGTATACTCGC
GGTGTCATGGATAGATGTGTATACTCGC

TTCCAGGAGTACCCTGATGAGATCGAGT
GCTCATGGGCACCGTGGCCAAAC..AGC
GCTCATGGGCACCGTGGCCAAAC..AGC
GCTCATGGGCACCGTGGCCAAAC..AGC
GCTCATGGGCACCGTGGCCAAAC..AGC

TGACGAGGGCCTGGAGTGTGTGCCCACT
TGACGATGGCCTGGAGTGTGTGCCCACT
TGACGATGGCCTGGAGTGTGTGCCCACT
TGACGATGGCCTGGAGTGTGTGCCCACT
TGACGATGGCCTGGAGTGTGTGCCCACT

Fig. 5(ii)

24/52

66302019661200

80
TCCACCATGCCAAGTGGTCCCAGGCTG.
CCGCCCAGGCCCCTGTCTCCCAGCCTGA
CCGCCCAGGCCCCTGTCTCCCAGCCTGA
CCGCCCAGGCCCCTGTCTCCCAGCCTGA
CCGCCCAGGCCCCTGTCTCCCAGCCTGA

160
GGATGTCTATCAGCGCAGCTACTGCCAT
G.....CTACCTGC.CAGCC.CCGGGAG
G.....CTACCTGC.CAGCC.CCGGGAG
G.....CTACCTGC.CAGCC.CCGGGAG
G.....CTACCTGC.CAGCC.CCGGGAG

240
ACATCTTCAAGCCATCCTGTGTGCCCCCT
TGGTGCCCAG.....CTGCGTGACTGT
TGGTGCCCAG.....CTGCGTGACTGT
TGGTGCCCAG.....CTGCGTGACTGT
TGGTGCCCAG.....CTGCGTGACTGT

320
GAGGAGTCCAACATCACCATGCAGATTA
GGGCAGCACCAAGTCCGGATGCAGATCC
GGGCAGCACCAAGTCCGGATGCAGATCC
GGGCAGCACCAAGTCCGGATGCAGATCC
GGGCAGCACCAAGTCCGGATGCAGA...

Fig.5(iii)

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321

VEGF165	TGCGGATCAAACCTCACCAAGGCC
SOM175	TCATGATCCGG...TACCCGAGCA
SOM175-e6	TCATGATCCGG...TACCCGAGCA
SOM175-e6&7	TCATGATCCGG...TACCCGAGCA
SOM175-e4

401

VEGF165	AAGAAAGATAG.....AGCAA
SOM175	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e6	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e6&7	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e4	AAAAAGGACAGTGCTGTGAAGCCA

481

VEGF165AAGCA.....
SOM175	CTCTGCCCCCGGAGCACCCCTCCCC
SOM175-e6
SOM175-e6&7
SOM175-e4	CTCTGCCCCCGGAGCACCCCTCCCC

561

VEGF165	A.....GATCCGCA
SOM175	GCACCACCAGCGCCCTGACCCCCG
SOM175-E6	GCACCACCAGCGCCCTGACCCCCG
SOM175-e6&7
SOM175-e4	GCACCACCAGCGCCCTGACCCCCG

641

VEGF165	TTGAGTTAAACGAACGTACTTGCA
SOM175	TAGAGCTCAACCCAGACACCTGCA
SOM175-e6	TAGAGCTCAACCCAGACACCTGCA
SOM175-e6&7
SOM175-e4	TAGAGCTCAACCCAGACACCTGCA

Fig.5(iv)

55040-1964E60

26/52

AGCACATAGGAGAGATGAGCTTCCTACA
GTCAGCTGGGGGAGATGTCCCTGGAAGA
GTCAGCTGGGGGAGATGTCCCTGGAAGA
GTCAGCTGGGGGAGATGTCCCTGGAAGA
.....

GACAAGAA....AATCCCTGTGG.....
GACAGGGCTGCCACTCCCCACCACCGTC
GATAG.....
GATAG.....
GACAGGGCTGCCACTCCCCACCACCGTC

.....
AGCTGACATCACCCATCCCCTCCAGCC
.....CC
.....
AGCTGACATCACCCATCCCCTCCAGCC

GACGTGTAAATGTTCTGCAAAAAC.AC
GACCTGCCGCTGCCGCTGCCGACGCCGC
GACCTGCCGCTGCCGCTGCCGACGCCGC
.....
GACCTGCCGCTGCCGCTGCCGACGCCGC

687

GATGTGACAAGCCGAGGCGGTGA
GGTGCCGGAAGCTGCGAAGGTGA
GGTGCCGGAAGCTGCGAAGGTGA
.GTGCCGGAAGCTGCGAAGGTGA
GGTGCCGGAAGCTGCGAAGGTGA

Fig.5(v)

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400

GCACAACAAATGTGAATGCAGACC...A
ACACAGCCAGTGTGAATGCAGACCTAAA
ACACAGCCAGTGTGAATGCAGACCTAAA
ACACAGCCAGTGTGAATGCAGACCTAAA
.....CCTAAA

480

.....GCCTTGCTCAGAGCGGAGA
CCCAGCCCCGTTCTGTTCCGGGCTGGGA
.....
.....
CCCAGCCCCGTTCTGTTCCGGGCTGGGA

560

.....TTTGTT.....TGTAC..A
CCAGGCCCCTCTGCCCACGCTGCACCCA
CCAGGCCCCTCTGCCCACGCTGCACCCA
.....
CCAGGCCCCTCTGCCCACGCTGCACCCA

640

AGACTCG..CGTTGCAAGGCGAGGCAGC
AGCTTCCTCCGTTGCCAAGGGCGGGGCT
AGCTTCCTCCGTTGCCAAGGGCGGGGCT
.....
AGCTTCCTCCGTTGCCAAGGGCGGGGCT

Fig.5(vi)

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<p><i>Fig 6(i)</i></p> <p>29/52</p>	<p><i>Fig 6(ii)</i></p> <p>30/52</p>
<p><i>Fig 6(iii)</i></p> <p>31/52</p>	

03249854 070800

VEGF ₁₆₅	M	N	F	L	L	S	W	V	H	W	S	L	A	L	L	L	Y	L	H	H	A	K	W	S	Q	A	A	P
SOM175 _{Short}	M	S	P	L	L	R	R	L	L	.	.	L	A	A	L	L	Q	L	A	P	A	Q	A	P
VEGF ₁₆₅	I	F	Q	E	Y	P	D	E	I	E	Y	I	F	K	P	S	C	V	P	L	M	R	C	G	G	C	C	N
SOM175 _{Short}	L	T	V	E	L	M	G	T	V	A	K	Q	L	V	P	S	C	V	T	V	Q	R	C	G	3	C	C	P
VEGF ₁₆₅	F	L	Q	H	N	K	C	E	C	R	P	K	K	D	R	A
SOM175 _{Short}	L	E	H	H	S	Q	C	E	C	R	P	K	K	K	D	S	A	V	K	P	D	R	A	A	T	P	H	H
VEGF ₁₆₅	C	K	C	S	C	K	N	T	D	S	R	C	K	A	R	Q	L	E	L	N	E	R	T	C	R	C	D	K
SOM175 _{Short}	H	A	A	P	S	T	S	A	L	T	P	G	P	A	A	A	A	A	A	D	A	A	S	S	V	A	K	
OR...																												
VEGF ₁₆₅	M	N	F	L	L	S	W	V	H	W	S	L	A	L	L	L	Y	L	H	H	A	K	W	S	Q	A	A	P
SOM175 _{Long}	M	S	P	L	L	R	R	L	L	.	.	L	A	A	L	L	Q	L	A	P	A	Q	A	P
VEGF ₁₆₅	I	F	Q	E	Y	P	D	E	I	E	Y	I	F	K	P	S	C	V	P	L	M	R	C	G	G	C	C	N
SOM175 _{Long}	L	T	V	E	L	M	G	T	V	A	K	Q	L	V	P	S	C	V	T	V	Q	R	C	G	G	C	C	P
VEGF ₁₆₅	F	L	Q	H	N	K	C	E	C	R	P	K	K	D	R	A
SOM175 _{Long}	L	E	H	S	Q	C	E	C	R	P	K	K	K	K	D	S	A	V	K	P	D	R	A	A	T	P	H	H
VEGF ₁₆₅	G	P	C	S	E	R	R	K	H	L	F	V	Q	D	P	Q	T	C	K	C	S	C	K	N	T	D	S	.
SOM175 _{Long}	P	R	C	T	Q	H	H	Q	R	.	.	P	D	P	R	T	C	R	C	R	C	R	R	R	R	S	F	L

Fig.6(i)

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M A E G G Q N H H E . V V K F M D V Y Q R S Y C H C P I E T L V D 60
 V S Q P D A P G H Q R K V V V S W I D V Y T R A T C C P R E V V P 55
 D E G L E C V P T E E S N I T M Q I M R I K P H Q G Q H I G E M S 121
 D D G L E C V P T E G Q H Q V R M Q I L M I R . Y P S S Q L G E M S 115
 R Q E N P C G P C S E R R K H L F . V Q D P Q T 170
 R P Q P R S V P G W D S A P G A P S P A D I T H P T P A P G P S A 175
 P R R
 G G A

191

207

M A E G G Q N H H E . V V K F M D V Y Q R S Y C H C P I E T L V D 60
 V S Q P D A P G H Q R K V V V S W I D V Y T R A T C C P R E V V P 55
 D E G L E C V P T E E S N I T M Q I M R I K P H Q G Q H I G E M S 121
 D D G L E C V P T E G Q H Q V R M Q I L M I R . Y P S S Q L G E M S 115
 R Q E N P S A P G A P S P A D I T H P T P A P G P L C 170
 R P Q P R S V P G W D S A P G A P S P A D I T H P T P A P G P L C 177
 R C K A R Q L E L N E R T C R C D K P R R 191
 R C Q G R L E L N P D T C R C R K L R R 222

Fig. 6 (ii)

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Homology of SOM175 to VEGF₁₆₅ is 27% (33%) at the protein level, however within this are blocks of 100% homology. In particular, many structural residues are conserved including those thought to be involved in homodimerisation of VEGF (by comparison with PDGF).
ie. Cysteine-17

Cysteine-47

Proline-70, Cysteine-72, Valine-74

Arginine-77, Cystein-78, Glycine-80, Cysteines-81 & 82
Cysteine-89, Proline-91
Cysteines 122 & 124

Fig. 6 (iii)

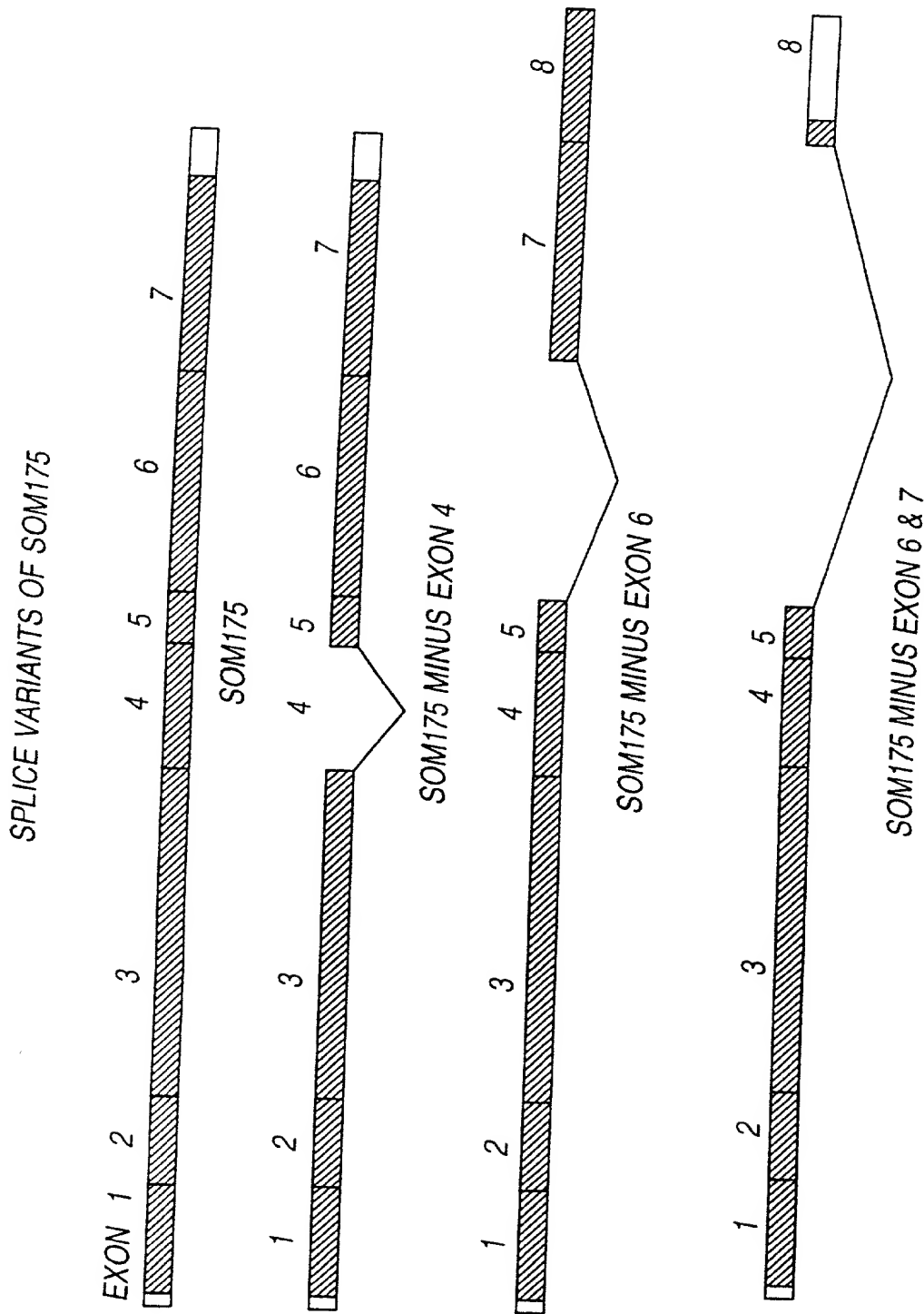


Fig. 7

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GENOMIC STRUCTURE OF HUMAN SOM175

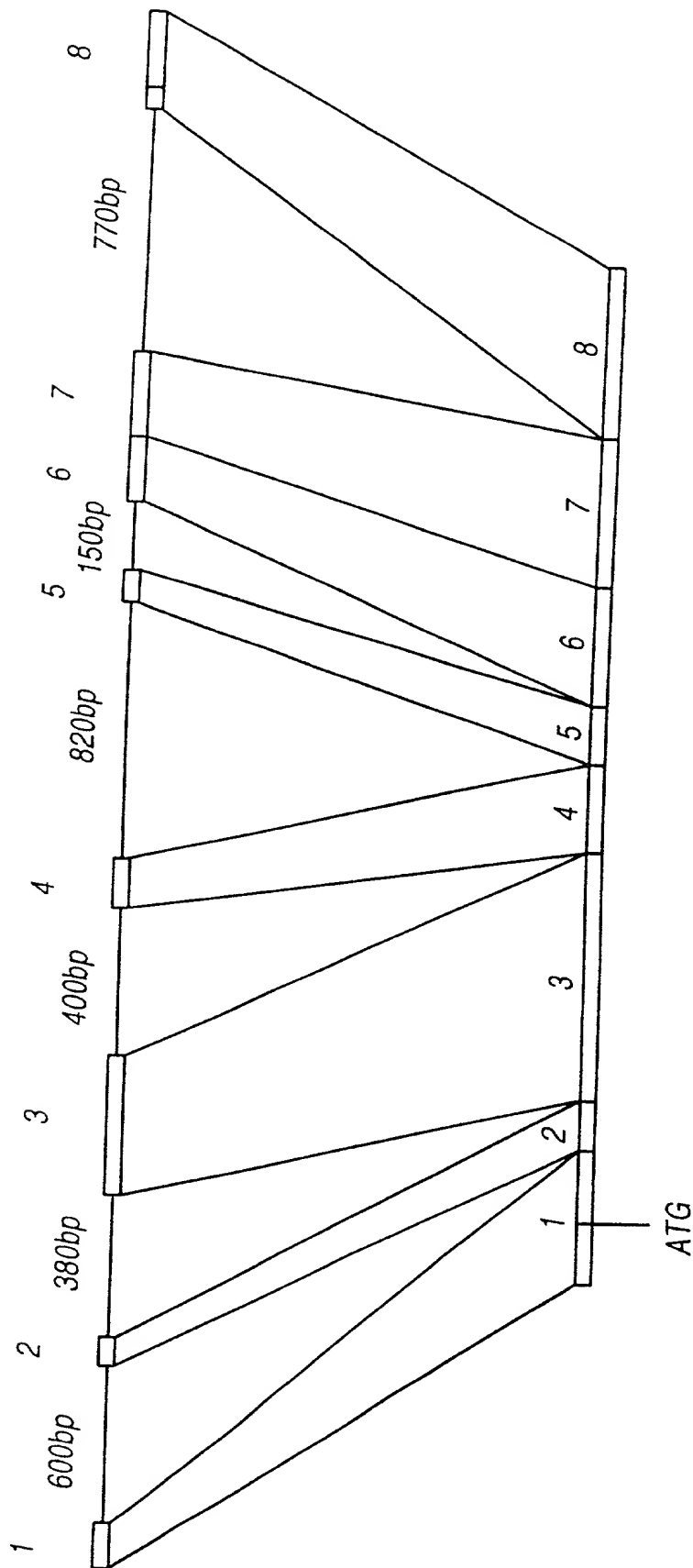


Fig.8A

34/52

5'UTR... ATGAGG	*Exon 1 (60bp)	GGCCAG gtacgtgagg
tctcccacag GCCCCT	Exon 2 (43bp)	GGAAAG aatacttaca
tctgctccca TGGTGT	Exon 3 (187bp)	ATGCAG gtccgagatg
ctgaatacag ATCCTC	Exon 4 (73bp)	ATGCAG gtgtcaggca
acttttcaag ACCTAA	Exon 5 (34bp)	AGACAG gtgagtcttt
ctcctccgta GGCTGC	Exon 6 (101bp)	CTCCAG cccaggccc
cccactccag CCCCAG	Exon 7 (109bp)	ACCCAG acacctgtag
ccctgctcag GTGCCG	*Exon 8 (22bp)	AGGTGA ...3'UTR

Fig.8B

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<p>36/52</p> <p>Fig. 9(i)</p>	<p>37/52</p> <p>Fig. 9(ii)</p>
<p>38/52</p> <p>Fig. 9(iii)</p>	<p>39/52</p> <p>Fig. 9(iv)</p>

63020 430400

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-163 gcacgagctcaggccgctcgctgcggcgctg
-103 gggggccgcggaggagccgccccctgcgcc
-43 ggcggtctctggctgacccccccccacaccg

16 CGTCGCCTGCTGCTTGTTGCACTGCTGCAG
R R L L L V A L L Q

76 TTTGATGGCCCCAGTCACCAGAAGAAAGTG
F D G P S H Q K K V

136 ACATGCCAGCCCAGGGAGGTGGTGGTGCCT
T C Q P R E V V V P

196 AAACAAC TAGTGCCCAGCTGTGTGACTGTG
K Q L V P S C V T V

256 GGCCTGGAATGTGTGCCCACTGGGCAACAC
G L E C V P T G Q H

316 TACCCGAGCAGTCAGCTGGGGGAGATGTCC
Y P S S Q L G E M S

376 CCTAAAAAAAAGGAGAGTGCTGTGAGGCCA
P K K K E S A V R P

436 CAGCCCCGCTCTGTTCCGGGCTGGGACTCT
Q P R S V P G W D S

Fig.9(i)

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cgttgcgctgcctgcgcccagggctcgggga
 ccgccccgggtccccgggtccgcgccatgg
 ccgggctagggcccgATGAGCCCCCTGCTG
 M S P L L -17
 ↓
 CTGGCTCGCACCCAGGCCCTGTGTCCCAG
 L A R T Q A P V S Q 4

 GTGCCATGGATAGACGTTTATGCACGTGCC
 V P W I D V Y A R A 24

 CTGAGCATGGAACATCATGGGCAATGTGGTC
 L S M E L M G N V V 44

 CAGCGCTGTGGTGGCTGCTGCCCTGACGAT
 Q R C G G C C P D D 64
 ↓
 CAAGTCCGAATGCAGATCCTCATGATCCAG
 Q V R M Q I L M I Q 84
 ↓
 CTGGGAGAACACAGCCAATGTGAATGCAGA
 L G E H S Q C E C R 104
 ↓
 GACAGGGTTGCCATACCCACCCGTCCTCC
 D R V A I P H H R P 124

ACCCCGGGAGCACCTCCCCAGCTGACATC
 T P G A P S P A D I 144

Fig.9(ii)

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496	ATCCATCCC <u>ACTCCAG</u> CCCCAGGATCCTCT
	I H P T P A P G S S
	S P R I L
556	CTGACCCCCGGACCTGCCGTTGCCGCTGTA
	L T P G P A V A A V
	P D P R T C R C R C
616	GGGGCTTAGAGCTCAACCCAGACACCTGTA
	G A *
	R G L E L N P D T C
676	ctttccagactccacgggcccggctgcttt
736	agcacaggcgtaacctcctcagtcctgggag
796	gagctctctcgccatctttttatctcccaga
856	atgtctcacctcaggggccagggtactctc
916	ttctggctggctgtctcccctcactatgaa
976	gggttctgttatgataactgtgacacacac
1036	gacactaaaaaaaaaaaaaaaaaaaaaaaaa

Fig.9(iii)

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G C C C G C C T T G C A C C C A G C G C C G C C A A C G C C
 A R L A P S A A N A 164
 C P P C T Q R R Q R 130

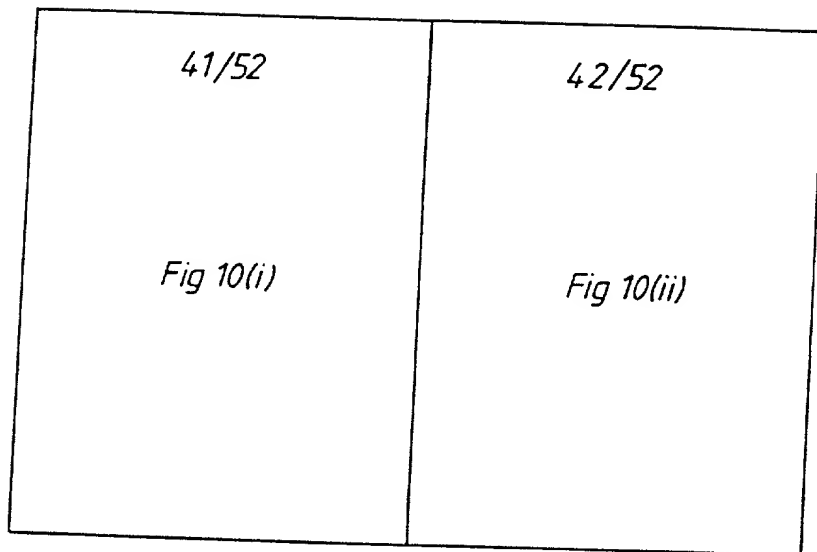
G A C G C C G C C G C T T C C T C C A T T G C C A A G G G C
 D A A A S S I A K G 184
 R R R R F L H C Q G 150

↓
 G G T G C C G G A A G C C G C G A A A G T G A c a a g c t g 186
 R C R K P R K * 167

t a t g g c c c t g c t t c a c a g g g a g a a g a g t g g
 g t c a c t g c c c c a g g a c c t g g a c c t t t t a g a
 g c t g c c a t c t a a c a a t t g t c a a g g a a c c t c
 t c a c t t a a c c a c c c t g g t c a a g t g a g c a t c
 a a c c c c a a a c t t c t a c c a a t a a c g g g a t t t
 a c a c a c t c a c a c t c t g a t a a a a g a g a t g g a
 a a a a a a a a a a a

Fig.9(iv)

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41/52

A

hVRF167 -21 MSPLLRRLLLAALLQLAPAQAP
 |||||
 mVRF167 -21 MSPLLRRLLLVALLQLARTQAP
 |||||

hVRF167 30 EVVVPLTVELMGTVAKQLVPSC
 ||||| : |||||
 mVRF167 30 EVVVPLSMELMGNVVKQLVPSC
 ||||| : |||||

hVRF167 80 ILMIRYPSSQLGEMSLEEHSQC
 ||||| : |||||
 mVRF167 80 ILMIQYPSSQLGEMSLGEHSQC
 ||||| : |||||

hVRF167 130 RPDPRTCRCRCRRRSFLRCQGR
 ||||| : |||||
 mVRF167 130 RPDPRTCRCRCRRRRFLHCQGR
 ||||| : |||||

B

hVRF186 116 RAATPHHRPQPRSVPGWDSAPG
 | |||||
 mVRF186 116 RVAIPHHRPQPRSVPGWDSTPG
 | |||||

hVRF186 166 TPGPAAAAADAAASSVAKGGA*
 ||||| : |||||
 mVRF186 166 TPGPAVA AVDAAASSIAKGGA*
 ||||| : |||||

Fig.10(i)

658020-1964E00

Fig. 10(ii)

186

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Temperature	25.0	°C	Pressure	1.0	atm
Time	1.0	h	Flow rate	1.0	ml/min
Concentration	0.1	g/l	Wavelength	254	nm
pH	7.0		Reagent	Merck	
Sample	Water		Standard	Merck	
Method	UV-Vis		Instrument	UV-1601	
Calibration	Linear		Software	UV-PRO	
Limit of detection	0.01	g/l	Limit of quantification	0.05	g/l
Recovery	95.0	%	Stability	98.0	%
Precision	2.0	%	Accuracy	99.0	%
Linearity	0.999		Robustness	99.0	%
Specificity	99.0	%	Interference	0.0	%
Storage	4.0	h	Repeatability	99.0	%
Stability	98.0	%	Reproducibility	99.0	%
Accuracy	99.0	%	Reliability	99.0	%
Robustness	99.0	%	Validity	99.0	%
Interference	0.0	%	Compliance	99.0	%
Repeatability	99.0	%	Documentation	99.0	%
Reproducibility	99.0	%	Reporting	99.0	%
Reliability	99.0	%	Archiving	99.0	%
Validity	99.0	%	Retention	99.0	%
Compliance	99.0	%	Access	99.0	%
Documentation	99.0	%	Security	99.0	%
Reporting	99.0	%	Backup	99.0	%
Archiving	99.0	%	Recovery	99.0	%
Retention	99.0	%	Disposal	99.0	%
Access	99.0	%	Transfer	99.0	%
Security	99.0	%	Integration	99.0	%
Backup	99.0	%	Interfacing	99.0	%
Recovery	99.0	%	Configuration	99.0	%
Disposal	99.0	%	Upgrade	99.0	%
Transfer	99.0	%	Migration	99.0	%
Integration	99.0	%	Deployment	99.0	%
Interfacing	99.0	%	Installation	99.0	%
Configuration	99.0	%	Operation	99.0	%
Upgrade	99.0	%	Maintenance	99.0	%
Migration	99.0	%	Support	99.0	%
Deployment	99.0	%	Training	99.0	%
Installation	99.0	%	Documentation	99.0	%
Operation	99.0	%	Reporting	99.0	%
Maintenance	99.0	%	Archiving	99.0	%
Support	99.0	%	Retention	99.0	%
Training	99.0	%	Access	99.0	%
Documentation	99.0	%	Security	99.0	%
Reporting	99.0	%	Backup	99.0	%
Archiving	99.0	%	Recovery	99.0	%
Retention	99.0	%	Disposal	99.0	%
Access	99.0	%	Transfer	99.0	%
Security	99.0	%	Integration	99.0	%
Backup	99.0	%	Interfacing	99.0	%
Recovery	99.0	%	Configuration	99.0	%
Disposal	99.0	%	Upgrade	99.0	%
Transfer	99.0	%	Migration	99.0	%
Integration	99.0	%	Deployment	99.0	%
Inter					

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mVRF167	-21	MSPLLRL..LLVALLQL..
		: : :
mVEGF188	-26	MNFLLSWVHWTLALLLYLHH
mVRF167	25	TCQPREVVVPLSMELMGNVV
		: : : : :
mVEGF188	24	YCRPIETLVDIFQEYPDEIE
mVRF167	75	QVRMQILMIQYPSSQ.LGEM
		: : :
mVEGF188	74	NITMQIMRIKPHQSQHIGEM
mVRF167	119ILCPPC
		:
mVEGF188	124	QKRKRKKSFRKSWSVHCEPC
mVRF167	152	GLELNPDTCRCKPRK
		: :
mVEGF188	173	QLELNERTCRCDKPRR

Fig.11(i)

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↓	AR.TQAPVSQFDGPSHQKKVVPWIDVYARA	24
	: : : : : : ::	
	AKWSQAAPTT.EGEQKSHEVIKFMDVYQRS	23
	KQLVPSCVTVQRCGGCCPDDGLECVPTGQH	74
	: : : : : ::	
	YIFKPSCVPLMRCAGCCNDEALECVPTSES	73
	SLGEHSQCECRPKKKESAVRPDSPR.....	118
	: :	
	SFLQHSRCECRPKKDRTKPEKKSVRGKGKG	123
	TQRRQR...PDPRTCRCRCRRRRFLHCQGR	151
	: : : : : :	
	SERRKHLFVQDPQTCKCSCKNTDS.RCKAR	172
		167
		188

Fig.11(ii)

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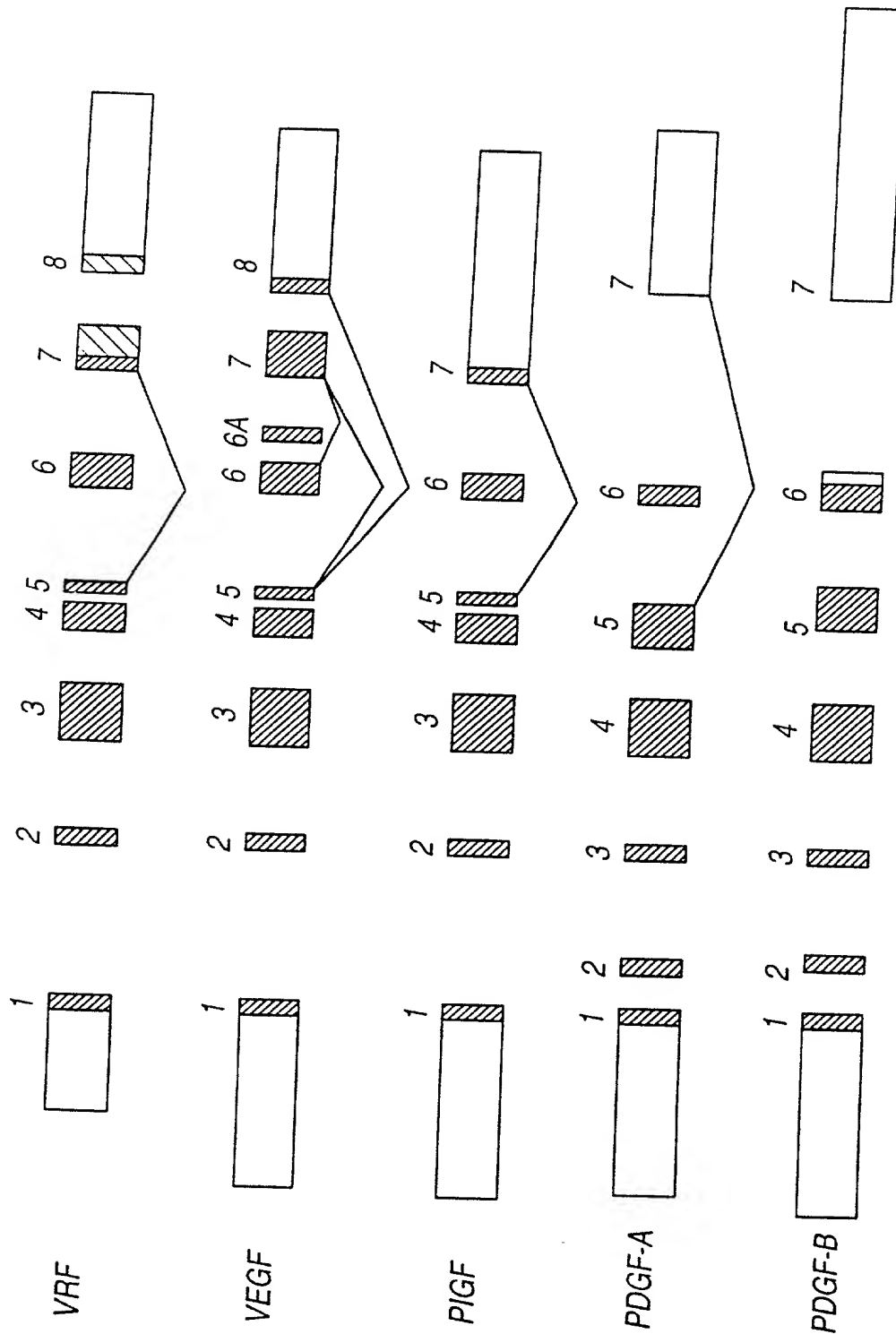


Fig.12

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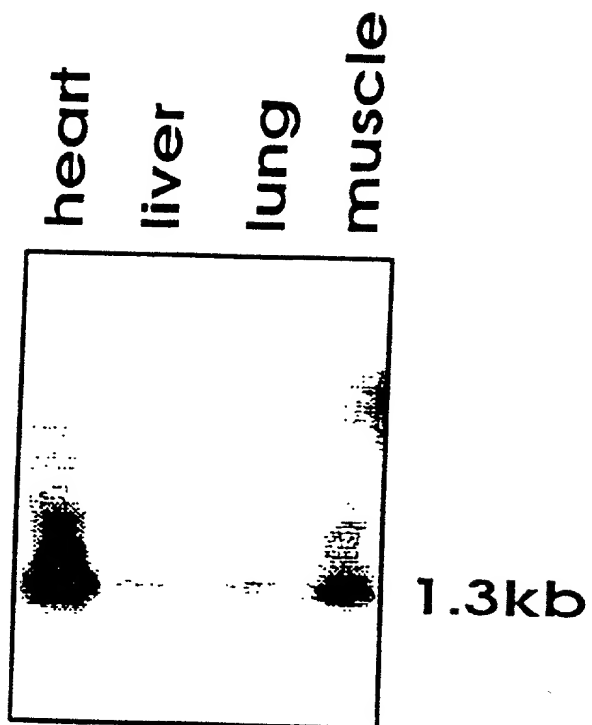


Fig.13

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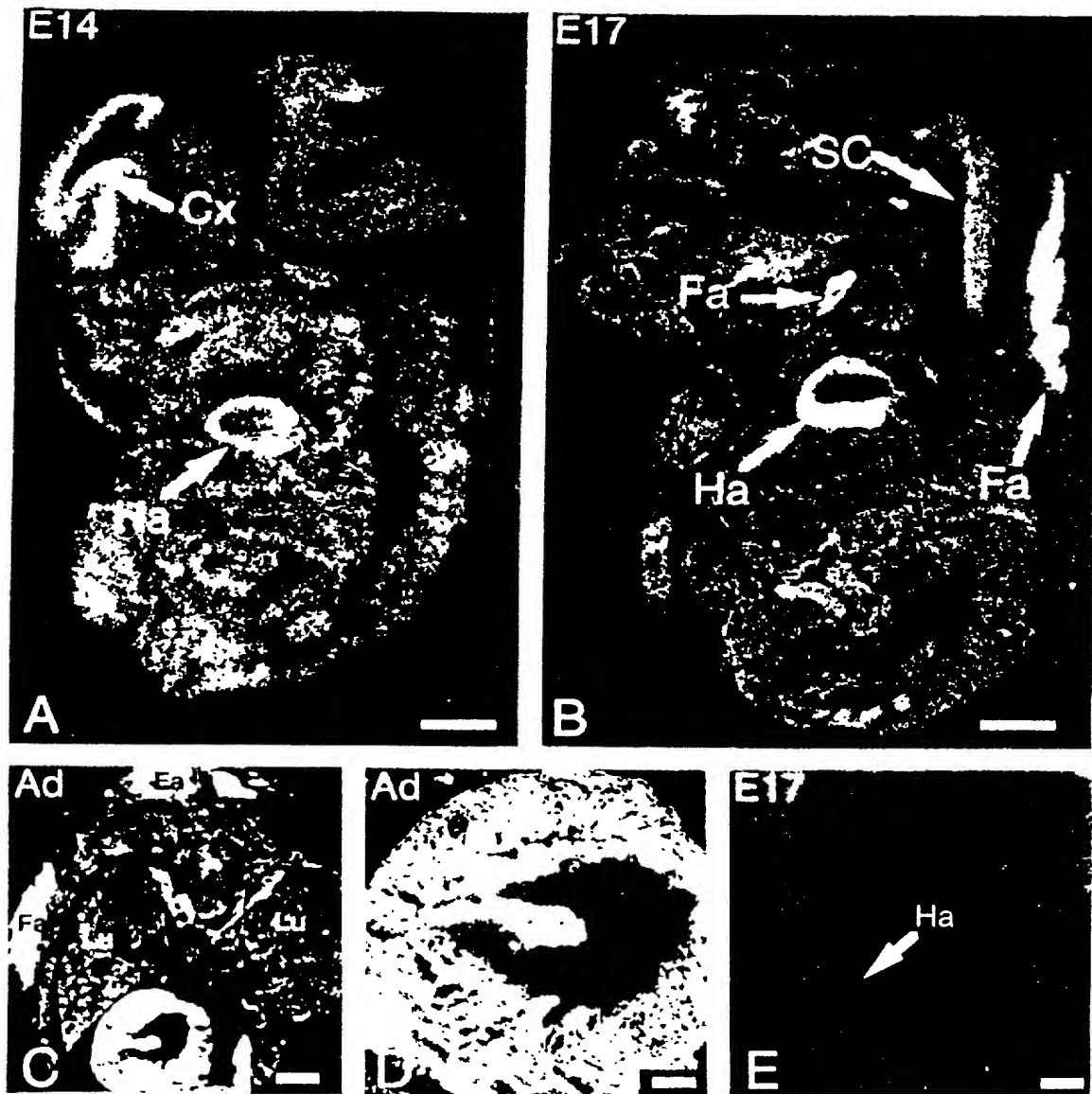


Fig.14

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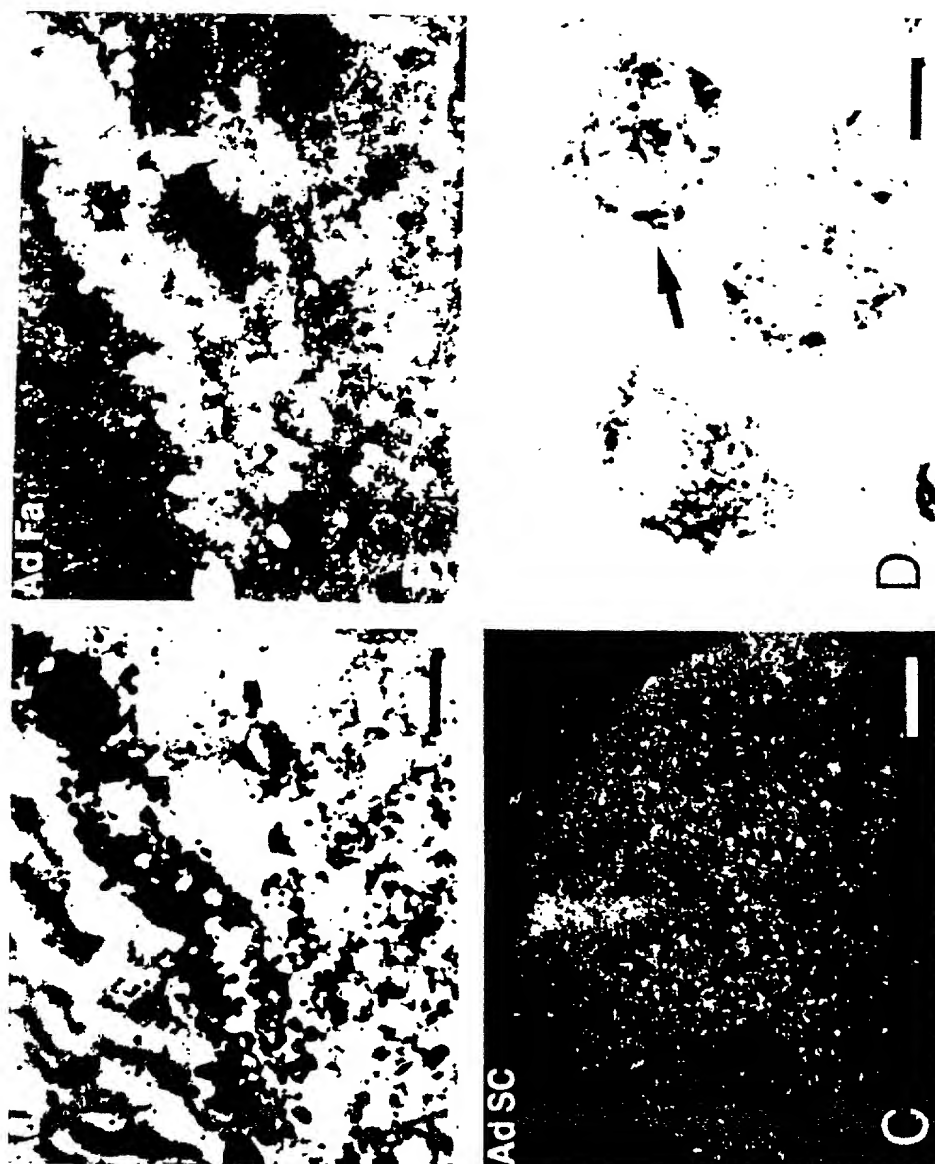


Fig. 15

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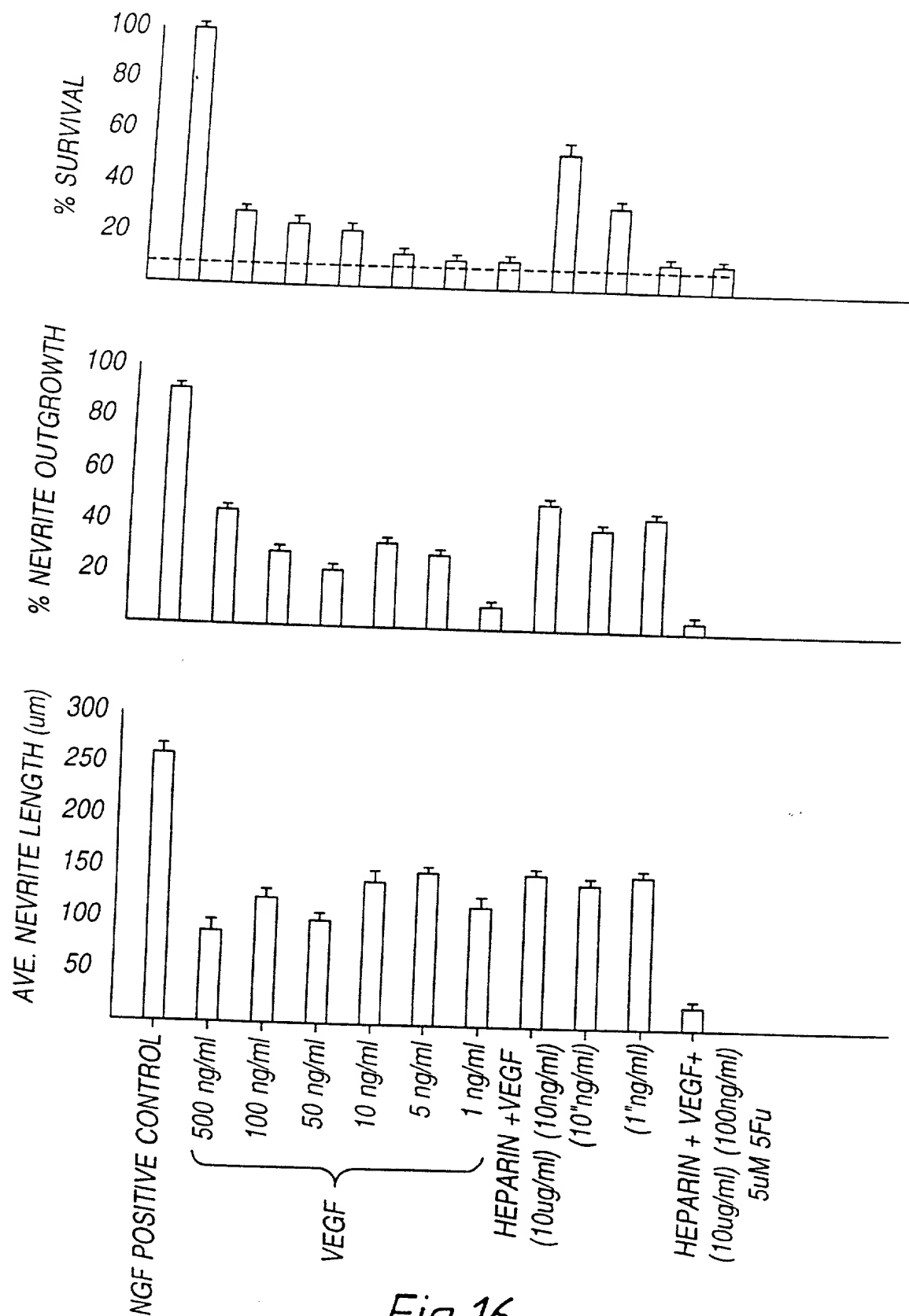


Fig.16

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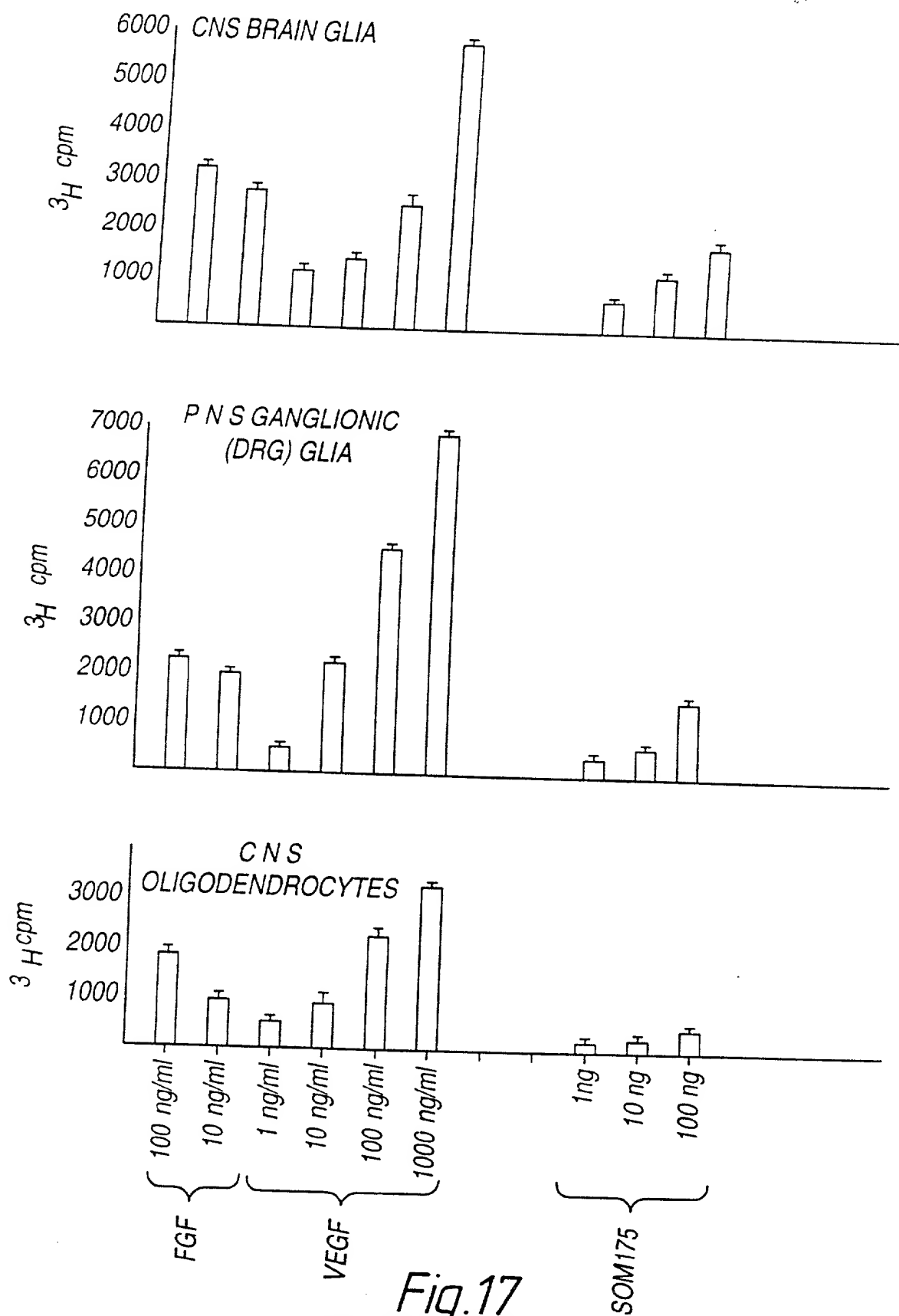


Fig.17

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MOUSE ASTROGLIAL CELLS

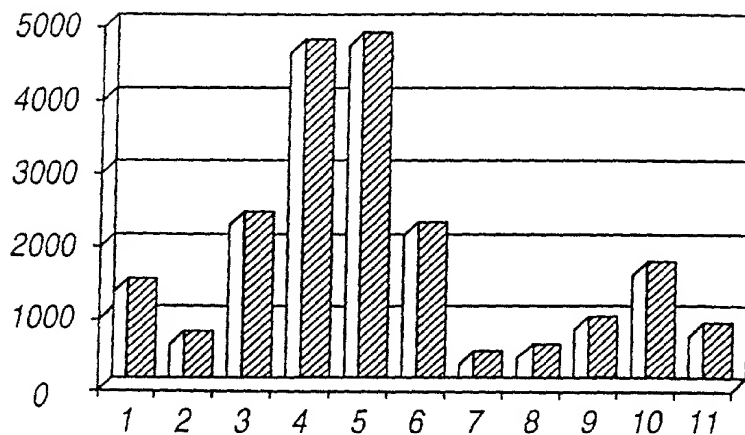


Fig.18

MOUSE OLIGODENDROGLIAL CELLS

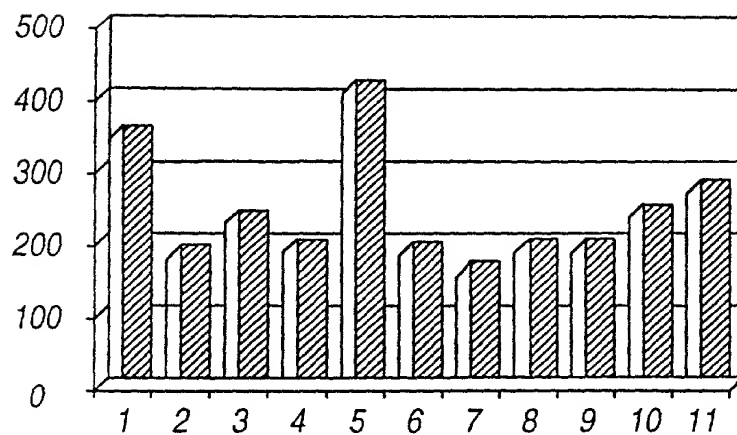
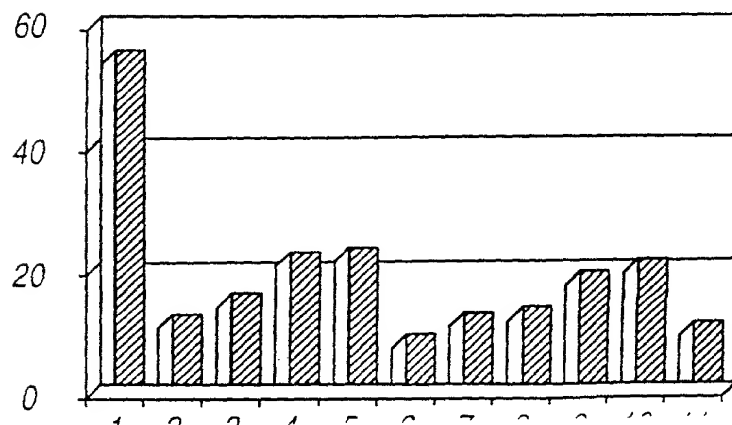


Fig.19

MOUSE FOREBRAIN NEURONS



Fin 20

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME"

the specification of which (check only one item below):

☒ is attached hereto.

☐ was filed as United States application

Serial No. 08/765,588

on 17 December 1996

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/AU96/00094

on 22 February 1996

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day month year)	PRIORITY CLAIMED UNDER 35 USC 119
Australia	PN 1457/95	2 March 1995	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Australia	PN 6647/95	20 November 1995	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Australia	PN 7274/95	22 December 1995	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

ATTORNEY S DOCKET NUMBER:

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

U S APPLICATIONS

STATUS (Check one)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. Stephen D. Murphy, Reg. No. 22,002; Leopold Presser, Reg. No. 19,827; William C. Roch, Reg. No. 24,972; Kenneth L. King, Reg. No. 24,223; Frank S. DiGiglio, Reg. No. 31,346; Paul J. Esatto, Jr., Reg. No. 30,749; John S. Sensny, Reg. No. 28,757; Mark J. Cohen, Reg. No. 32,211; Richard L. Catania, Reg. No. 32,608 and Donald T. Black, Reg. No. 27,999.

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(516) 742-4343

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 203

DATE _____

DATE 11th MARCH, 1997

[] Signature for fourth and subsequent joint inventors.
Number of pages added _____

602020 4469460

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	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
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SIGNATURE OF INVENTOR 207		SIGNATURE OF INVENTOR 208		SIGNATURE OF INVENTOR 209
DATE		DATE		DATE